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Steven J. Broderius
Lloyd L. Smith, Jr.

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University of Minnesota
St. Paul, Minnesota 55108

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The described analytical technique was used to define the relationship between test pH and sulfide toxicity to the fathead minnow. Within the pH range of 7.1 to 8.7, 96-hr LC50 values for molecular $H_2S$ decreased linearly from 57.3 to 14.9 ug/liter with increasing pH. However, the log 96-hr LC50 values of dissolved sulfide increased linearly from 64.0 to 780.1 ug/liter with increasing test pH ranging from 6.5 to 8.7.

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# EFFECT OF HYDROGEN SULFIDE ON FISH AND INVERTEBRATES

## Part II - Hydrogen Sulfide Determination and Relationship Between pH and Sulfide Toxicity

by

Steven J. Broderius
Lloyd L. Smith, Jr.
Department of Entomology, Fisheries, and Wildlife
University of Minnesota
St. Paul, Minnesota 55108

Grant No. R800992

Project Officer

Kenneth E. F. Hokanson
Environmental Research Laboratory - Duluth
Monticello, Minnesota 55362

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
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## ABSTRACT

An analytical method was developed for the direct determination of µg/liter concentrations of molecular $H_2S$. The procedure involves bubbling compressed nitrogen through an aqueous sulfide solution to displace $H_2S$ which is collected in a glass bead concentration column and measured colorimetrically. The $H_2S$ concentration is calculated from the determined sulfide displacement rate and by reference to a log linear standard curve relating temperature with the $H_2S$ displacement rate to the $H_2S$ concentration in standard solutions. To permit accurate determination of $H_2S$ from the determined dissolved sulfide concentration and fraction of dissolved sulfide as $H_2S$ for specific conditions of temperature and pH, the apparent linear relationship between $pK_1$ for $H_2S_{(aq)}$ and temperature was defined. This procedure of calculating $H_2S$ in various waters and effluents was confirmed by the direct technique.

The described analytical technique was used to define the relationship between test pH and sulfide toxicity to the fathead minnow. Within the pH range of 7.1 to 8.7, 96-hr LC50 values for molecular $H_2S$ decreased linearly from 57.3 to 14.9 µg/liter with increasing pH. However, the log 96-hr LC50 values of dissolved sulfide increased linearly from 64.0 to 780.1 µg/liter with increasing test pH ranging from 6.5 to 8.7.



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CONTENTS


| | | Page |
|---|---|---|
| Abstract | | iii |
| List of Figures | | vi |
| List of Tables | | vii |
| Acknowledgments | | ix |
| Sections | | |
| I | Conclusions | 1 |
| II | Recommendations | 2 |
| III | Introduction | 4 |
| | Theoretical Approach | 4 |
| | Literature Review | 7 |
| IV | Materials and Methods | 26 |
| | Determination of Sulfide in Aqueous Solution | 26 |
| | Direct Determination of Molecular $H_2S$ in Aqueous Solution | 36 |
| | Acute Sulfide Bioassays | 41 |
| V | Results and Interpretations | 46 |
| | Determination of Sulfide in Aqueous Solution | 46 |
| | Direct Determination of Molecular $H_2S$ in Aqueous Solution | 57 |
| | Equilibrium Constants for the First Dissociation of $H_2S_{(aq)}$ | 60 |
| | $H_2S$ Determination in Various Waters and Effluents | 67 |
| | Relationship Between Test pH and Sulfide Toxicity to the Fathead Minnow | 75 |
| VI | Discussion | 88 |
| | Determination of Molecular $H_2S$ and $K_1$ Ionization Constants of $H_2S_{(aq)}$ | 88 |
| | Modes of Toxic Action of Dissolved Sulfide to Fish | 89 |
| VII | References | 95 |
| VIII | Appendix | 103 |




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FIGURES


| No. | | Page |
|---|---|---|
| 1 | Relationship Between the First Dissociation Constant ($K_1$) of $H_2S_{(aq)}$ and Temperature for Aqueous Solutions of Generally Low Ionic Strength | 12 |
| 2 | Apparatus Used for Distribution Between Water and Nitrogen and Concentration of Molecular $H_2S$ | 37 |
| 3 | Relationship Between Test pH and Dissolved Sulfide, $HS^-$, and Molecular $H_2S$ Concentration at Levels Corresponding to the 96-hr LC50 for Fathead Minnows at 20 C | 87 |

TABLES


| No. | | Page |
|---|---|---|
| 1 | First Dissociation Constants of $H_2S_{(aq)}$ in Aqueous Solution at Various Temperatures and Generally Low Ionic Strength | 10 |
| 2 | Analysis of Laboratory Well Water | 42 |
| 3 | Linear Regression Analysis of Calibration Curves Relating Absorbance (Y) and Sulfide Concentration (X) in ug $H_2S$ per 25-ml for Solutions Prepared with Various Diamine Reagents and Under Different Acidity Conditions | 51 |
| 4 | Recovery and Stabilization of $H_2S$ by Glass Bead Concentration Columns Coated with Various Metal Salts | 53 |
| 5 | Stability of Metal Sulfides on Concentration Columns to Oxidation by Air | 55 |
| 6 | $H_2S$ Displacement by Nitrogen Dispersed Through Test Solutions of Known Molecular $H_2S$ Concentration and Temperature | 58 |
| 7 | Apparent $K_1$ Dissociation Constants and $pK_1$ Values of $H_2S_{(aq)}$ Determined for Test Solutions of Different Temperatures, pH Values, and Total Sulfide Concentrations | 62 |
| 8 | Relationship Between Apparent $K_1$ Dissociation Constants and $pK_1$ Values of $H_2S_{(aq)}$ for Temperatures Ranging from 10 to 25 C | 66 |
| 9 | Fraction of Dissolved Sulfide as Molecular $H_2S$ in Aqueous Sulfide Solutions of Low Ionic Strength | 68 |
| 10 | Multiplication Factors for Converting $H_2S$ Calculated from Pomeroy's Factors for a "Typical Water Supply" to Corresponding Concentrations Based on This Study | 72 |
| 11 | Multiplication Factors for Converting $H_2S$ Calculated from Factors in the 1946 to 1965 Editions of Standard Methods to Corresponding Concentrations Based on This Study | 76 |

| No. | | Page |
|---|---|---|
| 12 | Determination of Molecular $H_2S$ in Different Waters by Calculation from the Total and Dissolved Sulfide Concentration and by a Direct Technique | 78 |
| 13 | Determination of Molecular $H_2S$ in Different Effluents by Calculation from the Total and Dissolved Sulfide Concentration and by a Direct Technique | 80 |
| 14 | Summary of Test Conditions in Sulfide Bioassays at Different pH Values | 82 |
| 15 | Description of Fathead Minnows Used in Sulfide Bioassays at Different pH Values | 84 |
| 16 | Biological Assay by the BMD03S Probit Analysis Method of 96-hour Fathead Minnow Sulfide Bioassays Grouped According to Test pH | 85 |
| 17 | Summary of Lethal Concentration (LC) Analysis for 96-hour Fathead Minnow Sulfide Bioassays Grouped According to Test pH | 86 |

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## SECTION I

## CONCLUSIONS

1. The direct determination of molecular $H_2S$ in aqueous solutions at levels as low as 4 µg/liter can be accomplished by using a vapor phase equilibration technique in conjunction with a glass bead concentration column coated with 0.1 M zinc acetate.

2. The procedure of calculating molecular $H_2S$ concentrations in various waters and effluents from the determined dissolved sulfide concentration and the fraction of dissolved sulfide as $H_2S$ defined in this study, for specific conditions of temperature and pH, was confirmed by a direct method for $H_2S$ determination.

3. The 96-hr LC50 values of molecular $H_2S$ for the fathead minnow decreased linearly from 57.3 to 14.9 µg/liter with increasing pH within the range 7.1 to 8.7.

4. A positive linear relationship was observed between log dissolved sulfide concentration and test pH ranging from 6.5 to 8.7, at sulfide concentrations corresponding to the 96-hr LC50 values for the fathead minnow at 20 C.

5. The acute toxicity of sulfide solutions to fathead minnows does not depend entirely on the concentration of ambient molecular $H_2S$. The $HS^-$ ion appears to contribute to a much lesser extent to the toxicity of these solutions.

## SECTION II

## RECOMMENDATIONS

1. Research should be conducted to determine if the N,N-diethyl or N,ethyl-N-hydroxyethyl-p-phenylenediamine reagents should replace the N,N-dimethyl-p-phenylenediamine reagent generally used in the colorimetric determination of sulfide.

2. The factors corresponding to the decimal fraction of dissolved sulfide as molecular $H_2S$ proposed by Pomeroy[2] and those presented in the 9th through 13th editions of Standard Methods for the Examination of Water and Wastewater should be replaced by factors derived from the expression $pK_1 = 7.252 - 0.01342\ T\ (C)$ as defined in this study.

3. The best method for the determination of molecular $H_2S$, that has the widest application to practical situations, is based on calculations from dissolved sulfide, pH, and temperature measurements. It is recommended that this long-accepted procedure be continued and that use of the new proposed factors, sample preparation by filtration rather than flocculation, and optimization of the colorimetric test be employed.

4. Previous published $H_2S$ concentrations should be corrected to correspond to a common base derived from factors proposed in this study so that comparison of reported toxicity data will be consistent.

5. Reports of future sulfide toxicity tests in freshwaters should include molecular $H_2S$ concentrations and in addition the pH, temperature, and dissolved sulfide values.

## SECTION III

## INTRODUCTION

Hydrogen sulfide is one of the end products which may result from the bacteriological action on organic material containing protein and from various other chemical processes. This toxic substance is found in many ground and surface waters and numerous effluents are, either directly or indirectly, important sources of sulfides in natural waters. Because good toxicological information on sulfides with respect to aquatic species was lacking, the United States Environmental Protection Agency awarded a research grant (No. R800992) to the University of Minnesota to investigate the effect of hydrogen sulfide on various freshwater species and life history stages. The report on this project has been divided into two parts: I, dealing with the the toxic effects of hydrogen sulfide on aquatic organisms; II, dealing with the analytical determination of molecular hydrogen sulfide ($H_2S_{(aq)}$) and definition of the relationship between test solution pH and the toxicity of dissolved sulfide to the fathead minnow (Pimephales promelas Rafinesque).

### THEORETICAL APPROACH

The accuracy of the method used to calculate molecular $H_2S$ throughout Part I of this study was uncertain, suggesting the need for a method for the direct determination of $H_2S$. Such a method could then be used to define the relationship between $pK_1$ (i.e., $-\log K_1$) and temperature so that the current procedure of calculating $H_2S$ concentrations from dissolved sulfide, pH, and temperature measurements could be corrected.

An accurate means of determining molecular $H_2S$ could also be used to investigate the relative importance of $H_2S$ and the $HS^-$ ion in contributing to the acute toxicity to fish of sulfide solutions.

Determination of Molecular $H_2S$ and First Ionization Constant ($K_1$) of $H_2S$

It has become common practice to define total sulfide as dissolved $H_2S$ and $HS^-$, as well as acid-soluble metallic sulfides present in the suspended matter. The acid-insoluble sulfides such as copper sulfide and $S^{2-}$ ion, which is present in significant proportions only above about pH 11, are not included in this definition. The continued development of new and refinement of existing analytical methods for the determination of small quantities of sulfide suggests that no one method is entirely satisfactory or applicable to all types of samples. Determinations are also complicated by the fact that sulfides undergo oxidation in the presence of air or oxygen. The accepted procedure for determining molecular or un-ionized hydrogen sulfide in aqueous solutions is by appropriate calculations with the known concentration of dissolved sulfide, the pH of the sample, and the use of the first ionization constant ($K_1$) for $H_2S$. There are situations in which dissolved sulfide cannot be accurately determined with presently accepted analytical methods due to interferences or the presence of complex sulfides. The accuracy of the $H_2S$ determination depends, among other things, on the accuracy of the value assumed for the first ionization constant of $H_2S$ ($K_1$). A review of the scientific literature indicates that the relationship between $K_1$ and temperature is not well established and therefore the present method for $H_2S$ determination may be inaccurate.

Only through chemical analysis of test water can the relationship between concentration of the toxicant and the observed harmful effects on the test animals be definitely established. A specific and sensitive independent analytical method for the determination of undissociated molecular $H_2S$ which excludes other sulfide forms in water would be most useful. Such a method would be a tool to aid research concerning the toxicology of sulfides, for effective practical application of research

results to waste disposal control in natural waters, and prediction of the toxicity of water polluted with sulfide compounds. Therefore, a major objective of this study was to develop a direct analytical method to determine molecular $H_2S$ which would be accurate for concentrations as low as a few µg/liter. Its utility for the prediction or explanation of adverse conditions for aquatic life in natural and waste waters containing sulfide was also examined. Since the present acceptable method for calculating molecular $H_2S$ is applicable in most situations, a further objective of this study was to evaluate the relationship between $pK_1$ and temperature to permit accurate determination of $H_2S$ by this well established procedure.

The vapor phase equilibration method utilized during this study for the determination of molecular $H_2S$ does not significantly disturb equilibria involving other sulfides since less than 1 per cent of the dissolved sulfide is removed. In this procedure finely dispersed compressed nitrogen is bubbled continuously and at a regulated rate through a test solution to displace $H_2S$ which is trapped out of the nitrogen stream and quantified by a conventional colorimetric method for sulfide. The $H_2S$ concentration in the tested solution is obtained by reference to a log linear standard curve relating µg of $H_2S$ displaced per liter of nitrogen dispersed at various temperatures between 10 and 25 C to the known $H_2S$ concentration in standard $Na_2S$ solutions.

Test pH and Toxicity of Dissolved Sulfide

The $pK_1$ for $H_2S$ is approximately 7, thus at a pH value of near 7 about 50 per cent of the dissolved sulfide will be as $H_2S$ and 50 per cent as the hydrosulfide ion, $HS^-$. In more acidic solutions the equilibrium will shift towards a greater percentage of the sulfide as $H_2S$ and in more alkaline solutions towards that of the $HS^-$ ion. Therefore, in most natural waters the $HS^-$ ion can be expected to be the principal dissolved sulfide species. It is generally assumed that the toxicity of sulfide solutions to fish is mainly due to the penetration of the gills by the

undissociated $H_2S$ species and not of the $HS^-$ ion. Thus a change in test solution pH should have a drastic effect on the toxicity of a given concentration of dissolved sulfide. For any bioassay study it is necessary to know if the observed toxicity results from a specific toxicant known or believed to be present. Therefore, another objective of this research was to define the relationship between test pH and the toxicity of dissolved sulfide with special reference to the molecular $H_2S$ concentration. Continuous-flow 96-hr toxicity bioassays with fathead minnows were performed at six pH values to define this relationship and to test the validity of the assumption that the toxicity to fish of dilute sulfide solutions depends on the concentration of ambient molecular $H_2S$ with contribution of the $HS^-$ ion being negligible.

## LITERATURE REVIEW

### Equilibrium Constants for the First Dissociation of $H_2S_{(aq)}$

When $H_2S$ gas is dissolved in water an ionization equilibrium is established that can be represented by the equation:

$$H_2S_{(aq)} \underset{}{\overset{K_1}{\rightleftharpoons}} H^+ + HS^- \overset{K_2}{\rightleftharpoons} 2H^+ + S^{2-} \qquad (1)$$

The proportion of sulfur existing in aqueous solution as undissociated molecular $H_2S$ ($H_2S_{(aq)}$), hydrosulfide ion ($HS^-$), and sulfide ion ($S^{2-}$) is determined by the chemical and physical conditions in the solution and the equilibrium constants for the first and second dissociation of the sulfide species. The equilibrium expressions for the above reactions are given by:

$$K_1 = [H^+]\,[HS^-]/[H_2S_{(aq)}] \approx 10^{-7} \text{ at } 20\ C \qquad (2)$$

$$K_2 = [H^+]\,[S^{2-}]/[HS^-] \approx 10^{-13.5} \text{ at } 20\ C \qquad (3)$$

Using the approximate values of $K_1$ and $K_2$ at 20 C, it can be demonstrated

that the second equilibrium constant is so small that the percentage of dissolved sulfide as $S^{2-}$ ion is less than 0.32 per cent when the hydrogen ion concentration is greater than $10^{-11}$ (i.e., pH less than 11). Therefore, for practical purposes it is assumed that no significant amount of sulfide ion is formed below a pH of 11 and the second dissociation step and the presence of sulfide ion will be neglected in calculations and discussions in this report. The total concentration of dissolved sulfide species in solution is thus given by the concentration of molecular $H_2S$ ($H_2S_{(aq)}$) plus the hydrosulfide ion ($HS^-$). Theoretically then the concentration of molecular $H_2S$ in most freshwaters of low ionic strength (i.e., $\mu$ less than 0.01) can be calculated when the dissolved sulfide concentration, pH, and temperature are known and the relationship between the equilibrium constant for the first dissociation of $H_2S$ and temperature is defined. This procedure for the indirect determination of $H_2S$ is the proposed method of the American Public Health Association et al.[1] and has appeared in Standard Methods for the Examination of Water and Wastewater since 1946. This method is based on Pomeroy's[2] procedure for calculation of the un-ionized hydrogen sulfide concentration by multiplying the determined dissolved sulfide concentration by a factor representing the proportion of dissolved sulfide as molecular $H_2S$ at the pH, temperature, and ionic strength of the solution. It should be pointed out that in the APHA 1971 edition no relationship between $K_1$ and temperature was employed in this determination and no rationale for the use of the revised specified $K_1$ ($1.1 \times 10^{-7}$) at 25 C and ionic strength 0.02 was included.

Since about 1900 numerous investigators have employed a variety of techniques to measure the ionization constants of $H_2S$ at various temperatures. These values in general are not in good agreement but the first dissociation constant of $H_2S_{(aq)}$ given in the literature approximates $1 \times 10^{-7}$ at 25 C. Equilibrium constants vary with temperature and attempts to define this relationship over a range of temperatures occur in the chemical literature. Most values reported for $K_1$ by previous investigators are shown in Table 1. The single constant proposed by

Pomeroy[2] of $1.7 \times 10^{-7}$ at 25 C and $\mu = 0$, which has been used extensively, is considerably higher than values of $K_1$ at 25 C reported by other authors. This difference would suggest that Pomeroy's constant and data derived from it may be in error. A line defined by a linear regression equation depicting the relationship between $K_1$ and temperature from 0 to 35 C for most of the dissociation constants in Table 1 is shown in Figure 1. Thus the apparent equilibrium constant for the first dissociation of $H_2S_{(aq)}$ in aqueous solution of low ionic strength in relation to temperature (T) in degrees Celcius (C) is approximated by the expression:

$$K_1 = (0.31 + 0.029\ T) \cdot 10^{-7}. \qquad (4)$$

There have been a few attempts to define $K_1$ over a range of temperatures. Linear regression equations summarizing these findings are:

| Temperature range, C | Regression equation | Reference |
|---|---|---|
| 5 - 30 | $K_1 = (0.28 + 0.032\ T) \cdot 10^{-7}$ | Wright & Maass[6] |
| 10 - 35 | $K_1 = (0.33 + 0.020\ T) \cdot 10^{-7}$ | Tumanova et al.[7] |
| 0 - 25 | $K_1 = (0.27 + 0.024\ T) \cdot 10^{-7}$ | Loy & Himmelblau[4] |

The equation representing data from work by Loy and Himmelblau[4] defines a relationship between temperature and the true or absolute equilibrium constants since it includes corrections for experimental solution ionic strength. The earlier works of Wright and Maass[6] and Tumanova, Mishchenko, and Flis[7] represent relationships between the apparent equilibrium constant and temperature since corrections for ionic strength were not made. Barnes, Helgeson, and Ellis[5] summarize the work of Ringbom with the expression:

$$\log (K_1) = -7.05 + 0.0125\ (T - 25) \qquad (5)$$

Table 1. FIRST DISSOCIATION CONSTANTS OF $H_2S_{(aq)}$ IN AQUEOUS SOLUTION AT VARIOUS TEMPERATURES AND GENERALLY LOW IONIC STRENGTH

| Method | Temperature, C | Ionic strength, $\mu$ | $K_1 \cdot 10^7$ | Reference |
|---|---|---|---|---|
| Various | 0 | - | 0.1 | Jellinek & Czerwinski[3] |
| Radioactivity & conductance | 0 | 0 | 0.271 | Loy & Himmelblau[4] |
| Thermodynamics | 0 | - | 0.434 | Barnes et al.[5] |
| Conductance | 5 | 0 | 0.471 | Wright & Maass[6] |
| Thermodynamics | 5 | - | 0.501 | Barnes et al.[5] |
| Conductance | 10 | 0 | 0.574 | Wright & Maass[6] |
| Potentiometric | 10 | 0.02-0.04 | 0.534 | Tumanova et al.[7] |
| Thermodynamics | 10 | - | 0.579 | Barnes et al.[5] |
| Conductance | 15 | 0 | 0.747 | Wright & Maass[6] |
| Thermodynamics | 15 | - | 0.668 | Barnes et al.[5] |
| Conductance | 18 | - | 1.2 | Paul (1899) In: Pomeroy[2] |
| Conductance | 18 | - | 0.57 | Walker & Cormack (1900) In: Pomeroy[2] |
| Conductance | 18 | - | 0.91 | Auerbach[8] |
| Potentiometric | 18 | - | 3.31 | Epprecht[9] |
| Thermodynamics | 18 | - | 0.729 | Barnes et al.[5] |
| Conductance | 20 | 0 | 0.896 | Wright & Maass[6] |
| Potentiometric | 20 | - | 0.873 | Kubli[10] |
| Thermodynamics | 20 | - | 0.772 | Barnes et al.[5] |

Table 1 (continued). FIRST DISSOCIATION CONSTANTS OF $H_2S_{(aq)}$ IN AQUEOUS SOLUTION AT VARIOUS TEMPERATURES AND GENERALLY LOW IONIC STRENGTH

| Method | Temperature, C | Ionic strength, $\mu$ | $K_1 \cdot 10^7$ | Reference |
|---|---|---|---|---|
| Thermodynamics | 25 | - | 1.15 | Lewis & Randall[11] |
| Conductance | 25 | 0 | 1.08 | Wright & Maass[6] |
| Colorimetric | 25 | 0 | 1.7 | Pomeroy[2] |
| Colorimetric | 25 | 0.02 | 2.0 | Pomeroy[2] |
| Potentiometric | 25 | - | 1.24 | Yui[12] |
| Potentiometric | 25 | 0.02-0.04 | 0.790 | Tumanova et al.[7] |
| Spectrophotometric | 25 | 0 | 0.95 | Ellis & Golding[13] |
| Radioactivity & conductance | 25 | 0 | 0.87 | Loy & Himmelblau[4] |
| Thermodynamics | 25 | - | 0.891 | Barnes et al.[5] |
| Potentiometric | 25 | 0.10 | 1.6 | Hseu & Rechnitz[14] |
| Literature review | 25 | - | 0.955 | Chen & Morris[15] |
| Conductance | 30 | 0 | 1.26 | Wright & Maass[6] |
| Thermodynamics | 30 | - | 1.029 | Barnes et al.[5] |
| Potentiometric | 35 | 0.02 | 1.029 | Tumanova et al.[7] |
| Thermodynamics | 35 | - | 1.188 | Barnes et al.[5] |

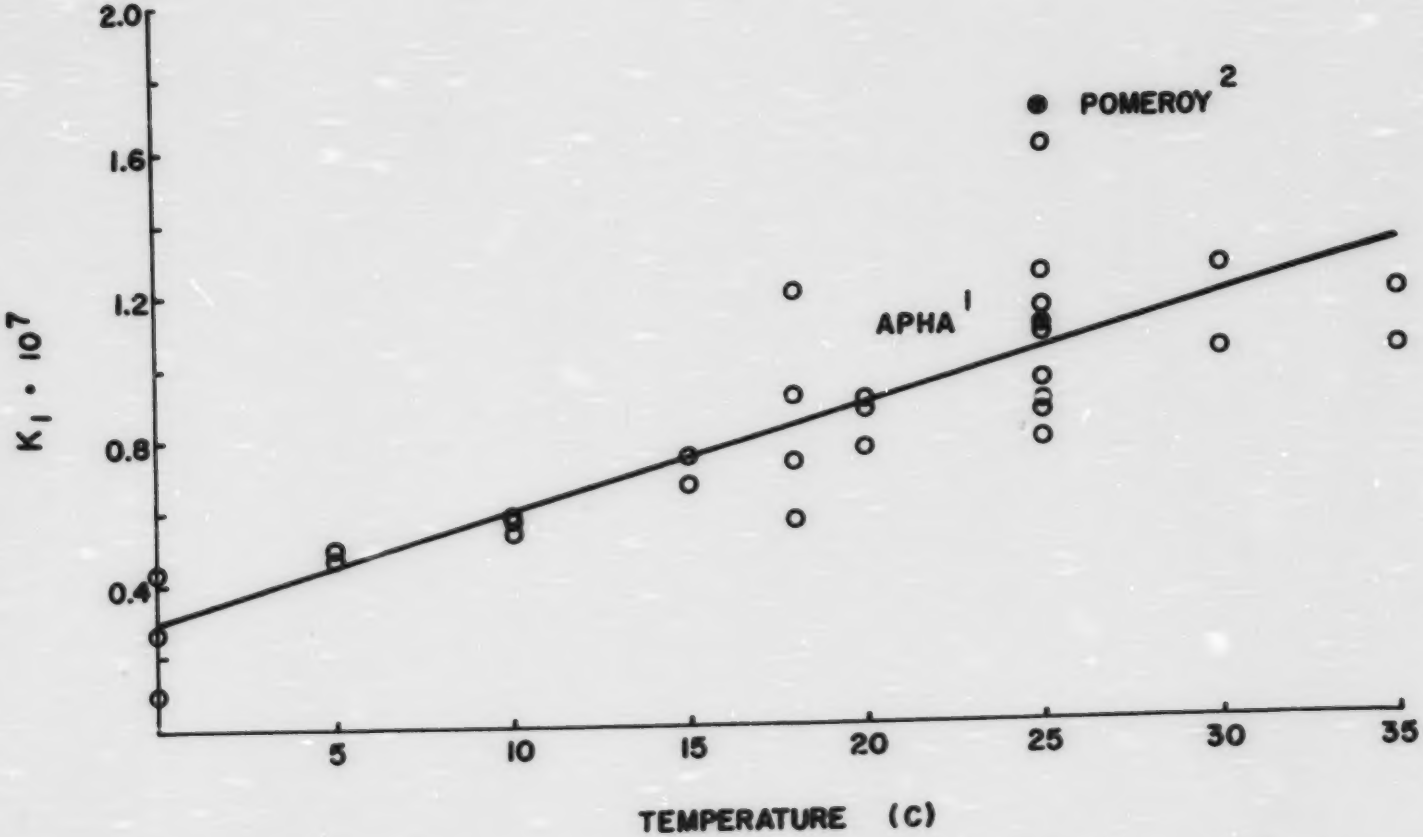


Figure 1. Relationship between the first dissociation constant ($K_1$) of $H_2S_{(aq)}$ and temperature for aqueous solutions of generally low ionic strength. Equation defining the regression line is $K_1 = (0.31 + 0.029\ T) \cdot 10^{-7}$.

where T = solution temperature in C.

Pomeroy[2] reported that if the effect of ionic strength on the activities of ions is considered when calculating the true or absolute ionization constant, the relationship can be expressed by:

$$\log (K_1) = \log [HS^-] - \log [H_2S] - pH - 0.5\sqrt{\mu} \qquad (6)$$

where $\mu$ = ionic strength.

He also reported that an increase in temperature has the same effect as an increase in pH to the extent that log $K_1$ increases by 0.0146 unit per degree centigrade.

Determination of Sulfide in an Aqueous Solution

The accurate determination of small amounts of sulfide is made difficult because sulfide is oxidized in the presence of air or oxygen. However, there are various quantitative methods to determine microgram quantities of aqueous sulfide and $H_2S$ evolved from sulfur and sulfides. These methods fall into three broad categories - volumetric using iodometric titration, colorimetric with the formation of colored complex compounds, and a fluorimetric procedure. Methods based on the isolation of evolved $H_2S$ usually employ alkaline or metallic solutions or suspensions as trapping agents.

Volumetric Iodometric Method—In most iodometric titration methods presented in the literature, dissolved and acid-soluble sulfides are oxidized to sulfur in an acid medium according to the following reaction:

$$H_2S + I_2 \longrightarrow S^o + 2H^+ + 2I^- \qquad (7)$$

There are many modifications of the titration method but they generally involve either titration of the sulfide directly with iodine or iodide-iodate mixture, or addition of an excess of iodine or iodide-iodate mixture and back titration with standard sodium thiosulfate.

Pomeroy[16] and Bethge[17] have outlined certain important sources of error affecting the iodometric titration method. Summarizing these findings, it can be stated that since $H_2S$ will escape an acidified solution, a moderately alkaline sodium sulfide solution should be pipetted directly into an acidified iodine solution, and the excess iodine back-titrated at room temperature with standard sodium thiosulfate to minimize the loss of $H_2S$. The iodine solution must be acidified to the proper extent since in alkaline solutions at room temperature iodine oxidizes a small portion of the sulfide to sulfate. It is also important that sulfides which are stabilized with an absorbant form acid-soluble precipitates and in addition, the ionic strength of the absorbant should be kept as low as possible. Also critical to this method is that the absorbant containing the sulfide be acidified before the addition of the iodine. Bethge[17] observed that if sulfide was added to a cadmium or zinc absorbant and the mixture in turn added to an iodine solution, the titrimetric results were highly variable. On the other hand if the absorbants without sulfide were added to the iodine solution, the amount of thiosulfate titrant consumed was comparable with the titration of iodine solutions alone. Apparently the large sulfur-containing particles formed in the presence of the metal absorbant include cadmium or zinc sulfide besides some iodine. Titrations should also be conducted allowing minimum contact of sulfide solutions with air and dissolved oxygen since sulfide is readily oxidized.

Bethge[18] discussed methods for the volumetric determination of sulfides in which the sulfide contained in a very strong alkaline or heated solution is oxidized by an excess of oxidant to sulfate. After oxidation, the excess oxidant is determined by adding potassium iodide and acidifying, and the iodine liberated is then back-titrated with sodium thio-

sulfate. Bethge[18] concluded that oxidation of sulfides to the sulfate state by potassium iodate is quantitative within experimental error. Therefore, his method forms a better basis for the estimation of sulfides than do methods employing the oxidants sodium hypochlorite and potassium permanganate, since these latter reagents are partially decomposed by boiling with strong alkali while potassium iodate is not. In methods in which the sulfide is oxidized to sulfate, four times as much oxidant is required, and so the sensitivity is increased fourfold over the methods in which sulfide is oxidized to sulfur.

Colorimetric Methylene Blue Method—The methylene blue reaction is recognized as one of the most specific and most sensitive of the few colorimetric procedures available for the determination of sulfides, allowing for the determination of approximately 10 µg/liter sulfide-sulfur. This method is based on the specific reaction of an acidic solution of N,N-dimethyl-p-phenylenediamine oxalate or sulfate (p-amino-N,N-dimethyl-analine) with sulfide in the presence of an excess amount of iron (III) oxidizing agent and chloride to cause complete color development of methylene blue in about 1 min. This is generally known as Lauth's or Caro's reaction. Following color development, diammonium hydrogen phosphate is usually added to eliminate the ferric color. Various combinations of reagents have been proposed for use in the color-forming reaction. The standard method proposed by APHA *et al.*[1] is based on Pomeroy's[16] modifications. Many applications of this method are reported in the chemical literature but since new modifications continue to appear, one should be aware that the method is reliable but lacks perfection.

Stoichiometry of reaction—If a stoichiometric reaction between sulfide and N,N-dimethyl-p-phenylenediamine is assumed, the reaction may be represented by 2 moles diamine reacting with 1 mole sulfide in the presence of ferric chloride to form methylene blue. According to the stoichiometric reaction, 1 mg sulfide should yield 9.97 mg dye. Pomeroy[18] observed that 1 mg sulfide produced 6.57 mg methylene blue for a

yield of 66 per cent. Therefore, it is evident that sulfide is not quantitatively transformed to methylene blue but that some is lost in side reactions. According to Gustafsson[19] the ferric ion together with N,N-dimethyl-p-phenylenediamine gives a red oxidation product, Wurster's red, which reacts in several ways with sulfide. Among other products, sulfide green, leuco methylene blue and methylene red are said to be formed. The first two are easily transformed to methylene blue, whereas methylene red is not. Methylene red and methylene blue are reported by Gustafsson[19] to be formed in the proportion 1:50. He also calculated that 66.7 $\pm$ 0.5 per cent of the added sulfide is transformed to methylene blue. Cline[20] stated that when a comparison is made of the apparent molar absorptivity with that of pure methylene blue solutions under comparable conditions the reaction is approximately 62 per cent complete. Recently, Zutshi and Mahadevan[21] observed the recovery of methylene blue from a given sulfide sample to be 65 $\pm$ 2.0 per cent of the theoretical value when using methylene blue obtained from different sources. When the methylene blue samples were dehydrated before preparing the solutions by keeping overnight in an oven at 80 C, the authors found that the recovery of methylene blue from sulfide samples was reduced to 51 $\pm$ 2.0 per cent. The loss in weight on drying is apparently due to loss of absorbed moisture by the methylene blue powder or crystals. This loss in weight was reported to be dependent on environmental conditions rather than the sources of manufacture.

Absorbance and molar absorptivity—According to Gustafsson,[19] Hofmann and Hamm,[22] Cline,[20] and Zutshi and Mahadevan[21] the color formed during the methylene blue reaction has an absorption spectrum with rather steep peaks and wave lengths of maximum absorption at about 670 and 745 nm. Hofmann and Hamm[22] stated that this maximum at 745 nm is not caused by the formation of a second dye but probably by the interaction between methylene blue and a chloroferrate (III) complex. The absorption at the peak wave lengths is strongly dependent on the concentration of acid in the mixture and as the pH of the color development solution declines, the primary absorption band of the formed methylene blue shifts

from about 670 to 745 nm.

Cline [20] and Grasshoff and Chan[23] concluded from their work and that previously reported by other authors that the final absorbance after dilution should be less than 1.0 in a 1-cm light path cuvette and preferably even less than 0.8 because aqueous methylene blue solutions do not strictly conform to Beer's Law at higher sulfide concentrations and that increasing the amount of reagents does not improve the linear range. Departure from Beer's Law was also observed by Cline[20] when the molar diamine concentration was less than seven times the total sulfide molar concentration. Dilution of more concentrated methylene blue solutions to a solution of exactly the same acid concentration permitted Johnson and Nishita[24] to restore the linear relationship and thereby prove that this lack of linearity is caused by deviations from Beer's Law and not by a decrease in the yield of methylene blue. Gustafsson[19] proposed an explanation for this observed phenomenon which depends on the assumption that the absorption spectrum of the formed methylene blue consists of two overlapping absorption bands with maxima at 600 and 650 nm. The former is more pronounced in more concentrated solutions and is due to the dimeric ion; the latter is due to the monomeric ion, predominating in very dilute solutions. The dissociation of the dimeric ion ($(MB)_2^{2+}$) is increased by diluting the solution or raising the temperature and thus increasing the relative absorbance at 670 nm.

The molar absorptivity index ($\epsilon$) of the methylene blue solution used by Johnson and Nishita,[24] corresponding to the range 5 to 50 µg sulfur in 100 ml, was about 34,500 liter $mole^{-1}$ $cm,^{-1}$ very close to that for maximum sensitivity of colorimetric methods. Grasshoff and Chan[23] concluded that the sensitivity of the methylene blue method is 0.2 µg at. $H_2S$-S/liter or 6.4 µg $H_2S$-S/liter.

Factors affecting color development—The effect of various factors such as reagent strength, acidity, temperature, light, and salt on the methy-

lene blue reaction have been investigated by numerous authors. It is well known that the absorbance of a methylene blue solution formed from a given amount of sulfide is pH-dependent. Gustafsson[19] observed that the acid concentration may affect both the yield of the reaction and the extinction of methylene blue. He reported that the yield of methylene blue increases with increasing acidity, whereas its extinction at 667 nm decreases. This result is different than the assumption made by Fogo and Popowsky[25] that variable acidity of the diamine reagent influences the absorption spectrum of methylene blue rather than the reaction yield. Tests by Grasshoff and Chan[23] showed that the absorbance of the methylene blue solution was essentially constant when the acidity was in the range 0.4 to 1.0 M. Gustafsson[19] reported that the optimum sulfuric acid concentration in the final solution is about 0.30 M when the reaction and measurement acidity are the same. Examination of his data also reveals that when the reaction acidity is about 0.67 M and the measurement acidity 0.1 M, an even greater absorbance at 667 nm is realized. In general, it can be stated that most studies have been confined to acid concentrations of less than 0.7 M and sulfuric acid has been found to be a better medium than hydrochloric acid for the diamine reagent.

Zutshi and Mahadevan[21] found that if the acid concentration during reaction and measurement is constant, only the 675 nm peak is observed at low acidity. This peak increases with increasing acidity, reaching a maximum at a sulfuric acid concentration of about 0.4 M, and gradually decreases at higher acidity. A peak also appears at 749 nm and increases in intensity with increasing acid concentration up to about 1.4 M, possibly indicating the formation of another compound produced by the addition of a proton to the methylene blue cation. At maximum extinction, the absorbance at this later peak is about 20 per cent greater than the maximum at the 675 nm peak. Zutshi and Mahadevan[21] proposed that the sum of the absorbances at both 675 and 749 nm or that at 749 nm alone be used to measure the sulfide concentration when the sulfuric acid concentration at both reaction and measurement is approximately 1.5 M. This suggestion is based on the fact that the arithmetic sum of

the two absorbance values remains more steady than the separate values when the acid concentration is varied. It was also stated that Beer's Law is very closely followed only at relatively high acid concentrations, a fact which may account for the deviations reported by earlier investigators who employed lower acid concentrations. Therefore, some care is necessary to maintain reproducible acid conditions in both standard solutions and actual determinations on samples so that a near optimal color development is realized with the specific sample volumes and dilutions employed.

The formation of methylene blue is not only a function of acidity but may depend on temperature. The reaction is known to be faster at higher temperatures, but at the same time the possibility of $H_2S$ escaping before reacting is increased. Gustafsson[19] stated that the precision of the method is much improved by keeping the temperature constant for both the reaction and the absorbance measurements. Zutshi and Mahadevan[21] found that at normal laboratory temperatures there is essentially no variation in the absorbance with temperature.

Patterson[26] stated that methylene blue solutions, although stable several days in the dark, fade rapidly in sunlight. However, Zutshi and Mahadevan[21] found that under normal laboratory lighting or in laboratory daylight there is no detectable photodecomposition effect on the methylene blue during or after the reaction.

The presence of many different electrolytes other than those added as reagents was found by Sands et al.[27] to have no effect on color intensity. Cline[20] stated that the method is without salt effect over the salinity range of 0 to 50 parts per thousand.

Interfering substances—The effect of a variety of substances on color development by the methylene blue procedure for sulfide has been investigated by various authors. Pomeroy[16] stated that sulfites, expressed as $SO_2$, or thiosulfate and chlorine up to concentrations of 10 mg/liter

have no serious effect on color development. By increasing the concentration of $FeCl_3$ and extending the time for reaction, accurate results may be obtained in the presence of 40 mg/liter $SO_2$ or $S_2O_3^{2-}$. Pomeroy[16] also stated that colloidal sulfur has no effect on the test. According to Sands et al.[27] carbon disulfide, the common types of organic sulfur compounds found in coke-oven gas, carburetted water gas, natural gas, and thiophene in concentrations about 40 times that of the sulfide do not appreciably affect color development. Pomeroy,[16] Sands et al.,[27] and Marbach and Doty[28] observed that many mercaptans produce a pink color on addition of the diamine reagent in the methylene blue method. However, this effect is essentially eliminated by addition of ammonium phosphate and by proper wave length selection, since the pink solutions do not significantly absorb near 670 or 745 nm. Because the methylene blue colorimetric assay is conducted in strongly acidic conditions, sulfides may be determined in the presence of many metals. Siegel[29] stated that $10^{-2}$ M zinc, cadmium, magnesium, and manganese do not interfere. Pomeroy[16] concluded that suspensions of iron, zinc, and lead sulfides react just as readily as dissolved sulfide. Copper, however, the sulfide of which possesses a solubility product of about $10^{-36}$, binds the sulfide so tightly that formation of methylene blue is almost completely inhibited.

Hyposulfite, $S_2O_4^{2-}$, and nitrite are two materials known to interfere with the methylene blue procedure. Hyposulfite gives a false positive sulfide test since it is decomposed by acid to $H_2S$ as one of the products. When nitrite is present in an acidified sample containing sulfide, some of the sulfide is oxidized to sulfur. Pomeroy[16] observed that a sample of sewage containing 1.0 mg/liter nitrite and 0.5 mg/liter sulfide produced a color corresponding to 0.4 mg/liter sulfide. In general, however, the methylene blue procedure is not seriously affected by most compounds which might ordinarily be expected to be present in natural waters, sewage, and many industrial effluents.

Diamine reagents—Zavodnov[30,31] stated that the reagents, p-phenylenediamine and N,N-dimethyl-p-phenylenediamine, used in the colorimetric

determination of sulfide in formation of methylene blue, can be replaced by N,N-diethyl-p-phenylenediamine or N-ethyl-N-hydroxyethyl-p-phenylenediamine without any procedural changes in the colorimetric determination of sulfide. Rees, Gyllenspetz, and Docherty[32] also investigated the use of various diamine reagents and showed that N,N-diethyl-p-phenylenediamine produced a stable ethylene blue color which was about twice as intense for a given amount of sulfide as that produced with the dimethyl diamine reagent normally used. The method employed was similar to the methylene blue procedure except that no diammonium hydrogen phosphate was added. This omission was made because the acidity of the final solution would not allow for the iron (III) phosphate (if present) to dissolve. The standard deviations at 100 and 1.0 µg sulfide per 100 ml were reported to be about 3 and 0.08 µg, respectively.

Isolation of $H_2S$—Paez and Guagnini[33] described a method for the isolation and ultramicro determination of $H_2S$ in gaseous mixtures or water by sorption on an anion-exchange resin column of hydroxide from Amberlite IRA 400, 20-50 mesh. The collected sulfide is then eluted with 4 M sodium hydroxide and determined colorimetrically by the methylene blue method. The technique was reported to permit the determination of 1 to 20 µg $H_2S$ present at dilutions of 0.07 to 20 ppm in air and down to 0.1 µg/liter in water. The sulfide can be kept on the resin without loss for as long as 10 days before the analysis. Many other investigators have employed alkaline or metallic solutions or suspensions as trapping agents in the isolation of evolved $H_2S$.

Fluorimetric Method—The analytical determination of $H_2S_{(g)}$ in air has received much attention in recent years. Jacobs, Bravermann, and Hochheiser[34] determined atmospheric $H_2S$ in the ppb range using the methylene blue method, but required large volumes of air, since either long sampling periods or high flow rates were necessary. The high flow rates resulted in low trapping efficiencies. Paper impregnated with Pb (II) and Hg (II) salts has been used extensively to trap $H_2S$. Sensenbaugh and Hemeon[35] used $Pb(OAc)_2$-impregnated paper traps, but this system could

be used only at low flow rates (<0.5 liter/min) and the PbS formed was unstable (Smith, Jenkins and Cunningworth[36]). Mercury (II) chloride forms the more stable HgS but, when combined with the methylene blue method is not sensitive enough to measure background levels (Hochheiser and Elfers[37]).

In order to measure $H_2S_{(g)}$ in the ppb range, an indirect fluorescence method involving fluorescein mercuric acetate (FMA) has been used. Andrew and Nichols[38] observed that fluorescein solutions in dilute aqueous alkali produced intense yellow-green fluorescence. Addition of sulfide to FMA solutions linearly decreases the fluorescence intensity, producing a pink coloration. The $S^{2-}$ - FMA reaction appears to be instantaneous. Axelrod et al.[39] observed that the fluorescence intensity was not affected by variations in ionic strength, but was affected by changes in pH. The FMA intensity was reported to fall off rapidly at NaOH concentrations greater than 0.15 N. However, NaOH concentrations between 0.05 and 0.1 N did not affect the FMA intensity, and 1.0 N $H_2SO_4$ added to neutralize any excess NaOH did not alter the fluorescence. The excitation and emission wave lengths generally utilized are about 499 and 519 nm, respectively. The useful range of analysis of sulfide is 0.5 to 10 x $10^{-8}$ M (0.16 to 3.2 µg-S/liter) in 0.1 N NaOH and 1 x $10^{-7}$ M FMA. It is important to note that the FMA reagent must be standardized daily with dilute sulfide solutions.

One of the most sensitive published methods for the determination of $H_2S_{(g)}$ in air is that of Axelrod et al.[39] who trapped the gas in an alkaline aqueous solution and estimated the resulting sulfide by the fluorimetric technique. While the sensitivity of this method is adequate, the collected sulfide is unstable (Avrahami and Golding[40]) and necessitates that analysis follow soon after sampling. Axelrod et al.[39] also attempted to place FMA in a bubbler to capture the $H_2S$ directly from the air. However, the FMA was strongly affected by aeration and by exposure to sunlight. Therefore, the FMA reagent should be added to the

sample following the collection and stabilization of sulfide. If precipitation is used to stabilize the sulfide, the precipitate must be redissolved prior to the addition of the FMA.

Natusch et al.[41] used a modification of the above method and were able to measure trace levels of atmospheric hydrogen sulfide as low as 5 parts per trillion (ppt, $10^{-12}$). According to their method $H_2S_{(g)}$ is extracted from air and stabilized as $Ag_2S$ by reacting with a $AgNO_3$-impregnated filter. This efficient recovery technique was first used by Smith et al.[36] The filters were prepared with Whatman No. 4 filter paper soaked for 2 min in 0.01 M $HNO_3$ containing 2% $AgNO_3$ and 2% ethanol and allowed to dry. The collected $Ag_2S$ is then dissolved with 0.1 M NaCN - 0.1 M NaOH solution producing the non-interfering silver cyanide complex $Ag(CN)_2^-$ and free sulfide which is analyzed fluorimetrically using very dilute fluorescein mercuric acetate. The advantages of the method include efficient collection of $H_2S$, good stability of the collected sulfide, and a sensitive and specific analysis method. Mercaptans and high ozone levels were reported to interfere with mercaptans reacting with $AgNO_3$ to form silver mercaptans which in turn quench FMA.

Vapor Phase Equilibration Method

Various methods have been published for the determination of dissolved gases in aqueous solutions but few can provide a precise measurement of existing concentrations since most methods involve marked disturbance of relevant equilibria through removal of all or a large proportion of the gas in question. The vapor phase equilibration procedure has proven to be a specific and sensitive analytical method for the direct determination of molecular HCN in aqueous solutions without material disturbance of existing ionic equilibria of the system. With this method a distribution equilibrium is established between the concentration of HCN in solution and in finely dispersed air or nitrogen bubbled through the sample. Analysis of the displaced HCN, which is collected on one of several possible types of concentration columns, can be accomplished by

various procedures. Schneider and Freund[42] used gas-liquid chromatography incorporating a thermal conductivity detector. Claeys and Freund[43] developed a more sensitive modification by utilizing a chromatographic procedure using a flame ionization detector while Nelson and Lysyj[44] measured the trapped HCN polarographically in a cell containing a stationary platinum cathode and rotating gold anode. The procedure developed and utilized in this investigation for the direct determination of molecular $H_2S$ most closely approximates in principle that described by Broderius,[45] where the displaced and collected HCN was determined by a colorimetric method. Once an accurate method was developed for the direct determination of molecular $H_2S$ a means of defining the relationship between $pK_1$ for $H_2S_{(aq)}$ and temperature was available. This ultimately would allow for the accurate calculation of $H_2S$ concentrations from dissolved sulfide, pH, and temperature measurements.

Toxicity of Sulfide Solutions in Relation to pH

Although detailed experimental work on the detrimental effect of $H_2S$ in the µg/liter range on many aquatic species and certain life history stages has been published in recent years, very limited work and only at high sulfide concentrations has been done to define the relationship between pH and toxicity of dissolved sulfide. Jacques[46] showed that the rate of sulfide penetration into cells of the alga Valonia macrophysa was proportional to the concentration of molecular $H_2S$ in the external solution. It was also demonstrated that after extended exposure in solutions of constant total dissolved sulfide, the equilibrium concentration of total sulfide inside the cells varied with external solution pH and by calculation can be shown to be proportional to the molecular $H_2S$ content of the test solutions. Therefore Jacques' work suggests that the rate of entrance and equilibrium concentration of sulfide in the cells is controlled by the diffusion of molecular $H_2S$ across the cell membrane.

Longwell and Pentelow[47] found that the acute toxicity of a standard

solution of 3,200 µg/liter total sulfide, as demonstrated by overturning time in minutes for 50 per cent of a brown trout sample, decreased as the pH increased from 6.0 to 9.0. This observed effect was attributed by the authors to the greater percentage of dissolved sulfide as free $H_2S$ at the lower pH values. Jones[48] also stated that the increased toxicity to sticklebacks of sulfide solutions at low pH values is most likely due to the greater proportion of the dissolved sulfide being present in the form of molecular $H_2S$. Bonn and Follis[49] measured the acute toxicity of sulfide from calculated molecular $H_2S$ concentrations to various life history stages of the channel catfish (Ictalurus punctatus) at pH values varying from 6.8 to 7.8. They observed that the toxic effects of sulfide appear to be independent of total sulfide in solution but are related to the calculated quantity of molecular $H_2S$ as controlled by the pH of the solution. This relationship was then applied by Bonn and Follis[49] in attempting to improve the productivity of acidic lakes having a high $H_2S$ level by raising the pH through addition of limestone. In summary, it appears that the toxicity of sulfide to fish may be largely attributed to the toxic action of molecular $H_2S$, varying with pH and the dissolved sulfide concentration in solution.

## SECTION IV

## MATERIALS AND METHODS

### DETERMINATION OF SULFIDE IN AQUEOUS SOLUTION

The purpose of this section is to describe the methods used throughout this research for the determination of sulfide. The procedures used included iodometric titration and colorimetric methods. As noted in the literature review, there are other methods which would permit the measurement of $H_2S$ at much lower levels but these two procedures were satisfactory for the purpose of this study since they are widely used, convenient, and allow for the determination of $H_2S$ at levels as low as a few µg/liter.

#### Iodometric Titration Method

This method is based on the addition of excess standard iodine solution to a sulfide solution under acidic conditions followed by titration with standard thiosulfate to determine the unreacted iodine and the iodine consumed by sulfide. Because hydrogen sulfide is volatile and sulfides are oxidized by dissolved oxygen, exposure to air and manipulation of the sulfide standard solutions was kept to a minimum. When a stabilizing absorbant was used the sulfide did not form an acid-insoluble metal precipitate and the absorbant containing the sulfide was added to an acidified solution before the addition of the iodine. The reagents in the titrimetric procedure were prepared with reagent grade chemicals and deionized water which had been boiled and allowed to cool and stored under a nitrogen atmosphere. Most of the reagents were prepared and standardized according to directions presented in the 13th edition of

Standard Methods (APHA[1]) under sections headed sulfide, titrimetric (iodine) method (section 228 A-3) and oxygen (dissolved), azide modification (section 218 B-2). The sodium thiosulfate titrant was standardized with both standard potassium biniodate and dichromate solutions with the determined average thiosulfate normality used in appropriate calculations.

Stock sulfide solutions were prepared by dissolving approximately 0.75 g sodium sulfide ($Na_2S \cdot 9H_2O$) in oxygen-free boiled, cooled deionized water. Crystals of the reagent grade sodium sulfide were rinsed free of oxidation products such as sodium sulfite and thiosulfate with deionized water, dried quickly on filter paper under an atmosphere of nitrogen, and weighed. The crystals are deliquescent but the rate of water absorption is slow. The weighed sodium sulfide crystals were immediately dissolved and diluted to 1.0 liter in a volumetric flask to form a solution containing approximately 0.1 mg S/1.0 ml. If the weight of $Na_2S \cdot 9H_2O$ used was not 0.75 g, the sulfide concentration was calculated from

$$\text{mg/liter S} = (133.4)\ B \qquad (8)$$

where S = sulfide concentration expressed as sulfur
B = g $Na_2S \cdot 9H_2O$/liter.

The stock sulfide solutions were standardized by adding approximately 100 ml deoxygenated deionized water to an Erlenmeyer flask, followed by 4 drops concentrated HCl. Then 10.00 ml standard iodine solution, followed immediately by 20.00 ml stock sulfide solution, were delivered to the flask below the solution surface. After 1 to 3 minutes, the residual iodine was determined by titration with sodium thiosulfate using starch indicator. The sulfide concentration, which should be approximately

100 mg/liter as S or 1 ml = 100 µg, is calculated as follows:

$$\text{mg/liter S} = \frac{(A-B)\ 16{,}000\ \mu\text{g/millieq.}}{\text{ml sample}} \qquad (9)$$

where S = sulfide concentration expressed as sulfur
A = (10 ml iodine) (milliequivalents iodine/ml)
B = (X ml $S_2O_3^{2-}$) (milliequivalents thiosulfate/ml).

Standard sulfide solutions were prepared from stock sulfide standards by standard techniques. These solutions are the working standards and were prepared immediately before use. Since there is a regular slow decrease in the sulfide concentration in stock sulfide solutions, the solutions were standardized as necessary before use. In some instances the sulfide in dilute working solutions was stabilized by diluting stock solutions with deoxygenated deionized water containing about 0.05 per cent w/v of zinc acetate.

Colorimetric Method

The principle of this method is based upon the formation of either methylene blue or ethylene blue by the action of one of a number of diamine reagents and ferric chloride on sulfide solutions in the presence of sulfuric acid. Ammonium phosphate is added to remove the color due to the presence of ferric ion before color intensity is spectrophotometrically measured. This reaction is highly sensitive but since the analysis is particularly sensitive to changes in certain conditions, strict adherence to details as set forth below is necessary for reproducible and accurate results.

The reagents in the colorimetric procedure were prepared with reagent grade chemicals and deionized water which had been boiled and allowed to

cool and stored under an atmosphere of nitrogen. Most of the reagents were prepared according to directions presented in Standard Methods (APHA[1]), under section headed sulfide, methylene blue visual color-matching method (section 228 B-3). Procedures for the preparation of reagents not included in this section are as follows:

Diamine-Sulfuric Acid Stock Reagent—Dissolve 14.78 g N,N-dimethyl-p-phenylenediamine oxalate, 17.06 g N,N-diethyl-p-phenylenediamine oxalate or 18.67 g N-ethyl-N-hydroxyethyl-p-phenylenediamine sulfate in a cold mixture of 50 ml concentrated $H_2SO_4$ and 20 ml deionized water; cool, then dilute to 100 ml with deionized water. The stock reagents are stored in dark glass bottles at 4 C. The oxalate solutions have been reported to be stable indefinitely while the sulfate solution is stable for at least a few months when stored at 4 C.

Diamine-Sulfuric Acid Reagent—Dilute 5.0 ml diamine-sulfuric acid stock solution with deionized water and 17.6, 57.7, or 77.7 ml concentrated $H_2SO_4$ to a final volume of 200 ml. These solutions were used in sulfide determinations when the desired reaction molar acidity was 0.25, 0.75, or 1.0, respectively. The solutions were stored in dark glass bottles at room temperature and have been reported to be stable for at least 1 month at 25 C.

The preparation of the diamine reagents and the amount used was done such that the concentration of diamine at the time of color development was the same as if the procedure outlined in Standard Methods (APHA[1]) had been followed. About 10 times as much diamine reagent was added as is theoretically necessary to react with 15 µg $H_2S$ in 25 ml.

The procedure for the colorimetric test employed in this study was designed to minimize the production of interfering colors, while quickly yielding near maximum blue color development. This end was accomplished by keeping the concentration of diamine relatively low, using a large excess of ferric chloride, and by adding diammonium hydrogen phosphate

after the blue color had developed. This latter reagent is important since it eliminates the yellow color of ferric chloride, the red color of the ferric chloride-diamine combination, and colors formed by certain interfering substances. The reagent also prevents further formation of the blue color and neutralizes enough of the acid to permit the near maximum blue color development. Color development is complete within a few minutes at room temperature and is stable for several hours if the solutions are kept in subdued light.

Standard sulfide solutions were prepared and immediately stabilized by forming an acid-soluble metal sulfide. The colorimetric determination can subsequently be completed either directly, or after proper dilution. This technique allows for color development in dilute sulfide solutions with negligible loss of the sulfide by evaporation or oxidation. Many different stabilizing solutions have been proposed but those which contain either zinc or cadmium have received the most attention. Several trapping solutions of these metals were tested to determine their collecting efficiency and stability of collected sulfide. The zinc stabilizing solutions that were used during this investigation were prepared as follows:

Zinc Acetate Sulfide-Absorbing Solution - 0.1 M Zn—Dissolve 21.95 g zinc acetate dihydrate ($Zn(OAc)_2 \cdot 2H_2O$) in deionized water. Acidify with about 3 drops of acetic acid to prevent hydrolysis and dilute to 1 liter.

Zinc Chloride Stabilizer Sulfide-Absorbing Solution - 0.1 M Zn—Dissolve 13.63 g zinc chloride ($ZnCl_2$) in deionized water, and 100 ml 1% (W/V) gelatin solution and dilute to 1 liter.

The cadmium stabilizing solutions that were used during this investigation were prepared as follows:

Alakline Cadmium Hydroxide Sulfide-Absorbing Suspension - 0.017 M Cd—

Dissolve 4.3 g cadmium sulfate ($3CdSO_4 \cdot 8H_2O$) in deionized water. Add 0.3 g sodium hydroxide, dissolved in water, and dilute to 1 liter. The final pH of the suspension was 7.7 and the suspension was mixed well each time before using.

Cadmium Chloride Sulfide-Absorbing Solution - 0.1 M Cd—Dissolve 22.84 g cadmium chloride ($CdCl_2 \cdot 2\text{-}1/2\ H_2O$) in deionized water and dilute to 1 liter.

Cadmium Sulfate Sulfide-Absorbing Solution - 0.1 M Cd—Dissolve 25.65 g cadmium sulfate ($3CdSO_4 \cdot 8H_2O$) in deionized water and dilute to 1 liter.

Calibration curves defining the relationship between absorbance and µg sulfide expressed as $H_2S$ in a total volume of 25 ml were prepared for later reference. The standard sulfide solutions were prepared by diluting, with freshly boiled and cooled deionized water, a stock sodium sulfide solution whose sulfide concentration was determined iodometrically. The curves were made immediately after the preparation of the sulfide standard solution. Aqueous standards were prepared by adding to separate 25-ml volumetric flasks, each containing 0.5 ml of a sulfide-stabilizing absorbant, the following volumes of a dilute aqueous sulfide solution containing approximately 2.5 µg $H_2S$/ml: 0 (reagent blank), 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml, in order to prepare a sulfide series containing approximately 0, 1.25, 2.5, 5.0, 7.5, 10.0, and 12.5 µg $H_2S$, respectively. Portions of the standard solution were transferred to the volumetric flasks with a pipette, submerging the tip in the absorbant before releasing the solution to avoid loss of sulfide. The standard sulfide solutions were then diluted in each flask with boiled and cooled deionized water to a volume of 5.5 ml. In all instances the standards and samples were contained in the same volume before adding colorizing reagents.

Color development is then accomplished by adding either 0.9 ml diamine-sulfuric acid reagent, rapidly followed by the addition of 0.1 ml ferric

chloride reagent, or 1.0 ml fresh diamine and iron solution, prepared by mixing nine parts of the diamine reagent with one part of the ferric chloride solution. After at least 1 min, and following mixing, 1.5 ml diammonium hydrogen phosphate solution is added to eliminate the ferric chloride color and the solution is mixed to dissolve the white ferric phosphate precipitate. Then the solutions are diluted to volume with deionized water and the flasks are stoppered. Reasonable care was taken to use the specified amounts of reagents. However, it was observed that moderate variation with reagent concentrations would not markedly affect color development. The relative amounts of acid and ammonium phosphate must be balanced in such a way that the ferric phosphate will remain in solution at the end of the test.

The temperature of the solutions in the flask while adding reagents and during absorbance measurements was between 19 and 21 C. Following dilution to volume, the solutions were allowed to stand for about 30 min in subdued light and then measurement of the absorbance in a 1-cm light path cuvette was made using a Beckman DB-GT spectrophotometer against the reagent blank, which is nearly colorless. The wave lengths at which absorbance measurements were made are 666, 668, and 672 nm for solutions prepared with the N,N-dimethyl-p-phenylenediamine oxalate, N-ethyl-N-hydroxyethyl-p-phenylenediamine sulfate, and N,N-diethyl-p-phenylenediamine oxalate color developing reagents, respectively.

After completing one series of standards, the working sulfide standard solution was discarded, a new standard solution was prepared and a second set of standards were analyzed. This procedure was repeated several times and the relationship between micrograms of sulfide used in preparing the solutions (expressed as $H_2S$) and average blank-corrected absorbance values for the individual diamine reagents were defined by linear regression equations.

## Determination of Total and Dissolved Sulfide

The distinction between total and dissolved sulfide can be made by a number of different procedures. During this research total sulfide was determined either by employing a direct colorimetric measurement on zinc acetate-stabilized sulfide or by using a volatilization procedure followed by adsorption.

Sulfide solutions to be used for direct colorimetric measurement were stabilized by adding approximately 30 ml of the test solution, followed by a few drops of $Na_2CO_3$ solution when necessary to increase pH, to a glass vial containing four drops of 2.0 N zinc acetate. Color development on a well mixed 7.5-ml aliquot of the sample was then accomplished according to directions presented in Standard Methods (APHA[1]), under the section headed methylene blue visual color-matching method (section 228 B-4a(2)). Color intensity is determined with a spectrophotometer and $H_2S$ calculated from an appropriate calibration curve.

The volatilization procedure involves stripping $H_2S$ from an acidified solution by a stream of nitrogen. The evolved sulfide is carried over and quantitatively absorbed as zinc sulfide on a glass bead concentration column coated with 0.1 M zinc acetate. The metal sulfide produced is then determined colorimetrically. The determination of total sulfide is accomplished by addition of between 50 and 200 ml of sample to a 300-ml three-neck distilling flask containing 1.0 ml of 0.1 M zinc acetate solution. The volume of sample in the flask is determined by difference in weight before and after the addition of sample and from the density of water at 20 C. The flask is then connected to a spray trap by means of a standard taper (i.e., [illegible]) ground glass joint. A dropping funnel containing 1 + 1 $H_2SO_4$ is fitted in the middle neck of the distilling flask by means of a [illegible] ground glass joint. The glass bead concentration column, coated with approximately 0.5 ml of 0.1 M zinc acetate absorbant, is then placed in series with the spray trap and connected with an O-ring joint. A coarse porosity glass frit extending to

the bottom of the flask is inserted in the third neck and connected with a ▮ ground glass joint. A nitrogen delivery tube is connected to the frit by means of an O-ring joint. Nitrogen is then allowed to pass through the system to displace all oxygen before releasing the stabilized sulfide. The rate of nitrogen flow through the apparatus is adjusted to approximately 300 ml/min. This flow is sufficiently rapid to sweep the system completely free of $H_2S$ in less than 60 min. Following this procedure the stopcock in the dropping funnel is opened and 20 drops of 1 + 1 $H_2SO_4$ is allowed to run slowly into the mixture, but the flow is stopped so that some acid remains in the bulb. The acid converts the dissolved $HS^-$ and $S^{2-}$ ions as well as acid-soluble metallic sulfides to $H_2S$ because of the reduction in sample pH. The steady stream of nitrogen is maintained for 1 hr through the system after acidification. During the stripping reaction no heat was applied other than that produced by addition of acid. The $H_2S$ displaced from the sample by the stream of nitrogen is then adsorbed on a glass bead concentration column as a metal sulfide precipitate. The displaced sulfide is at room temperature and is protected from exposure to light during and following collection until analysis. Samples generally contained between 2 and 10 µg $H_2S$ and were analyzed as soon as possible after collection to avoid loss of sulfide. It should be emphasized that air was excluded at all stages to prevent oxidation and the escape of gaseous $H_2S$.

The precipitate in the concentration column is treated with 5.0 ml of deionized water, 0.9 ml of the acidic N,N-dimethyl-p-phenylenediamine reagent, and mixed by inversion. Then 0.1 ml of the iron (III) reagent is added to the column, sealed with parafilm, and mixed by inversion. The $H_2S$ evolved immediately reacts with the resultant formation of methylene blue. The $H_2S$ which escapes into the air space above the liquid is minimal since the liquid in the concentration column comprises essentially the entire volume. After at least 2 min the colorized solution in the concentration column is quantitatively removed into a 25-ml volumetric flask with three 5-ml washings of deionized water. After approxi-

mately 30 min the absorbance of the resulting methylene blue solution is measured at 666 nm with a 1-cm light path cuvette and against a reagent blank. The corresponding quantity of sulfide expressed as µg $H_2S$ is then determined from previously prepared calibration curves developed from similar measurements on solutions prepared by adding a known amount of sulfide directly to 25-ml volumetric flasks. Since absorbancy of methylene blue solutions is influenced by acid concentration, care was exercised in maintaining acid concentrations constant in all determinations.

Dissolved sulfide was determined on samples following removal of suspended solids by flocculation and settling, centrifugation, and filtration. The flocculation of suspended solids was accomplished by filling a 125-ml reagent bottle to overflowing with test solution, followed immediately by the addition of 0.2 ml of 6 N aluminum chloride solution and 2.2 ml of 6 N NaOH. The bottle was stoppered to exclude air and then rotated back and forth about a transverse axis in order to flocculate the contents. The reagents used were prepared according to directions presented in Standard Methods (APHA[1]) under the section headed titrimetric (iodine) method (section 228 A-3). The flocculant was allowed to settle for 15 min and then a portion of the clear supernatant was removed and stabilized with zinc acetate. The removal of suspended solids was also accomplished by filling a 30-ml vial to overflowing with test solution, capping, and centrifuging for 10 min. A portion of the supernatant was then removed and stabilized with zinc acetate. Removal of the suspended matter by filtration was accomplished by drawing a 20-ml sample into a 20-ml glass syringe, excluding as much air as possible, and forcing the sample through a 0.45-micron millipore filter of 25 mm diameter utilizing a swinny-type filter holder adapted by swedge lock connection with the hypodermic syringe. The filtrate was stabilized immediately with zinc acetate. Whenever the pH of the test solutions was below about 7.5, two drops of 5% $Na_2CO_3$ were added to the zinc sulfide solution to insure sulfide stabilization. Color development and sulfide determination on a well mixed 7.5-ml aliquot of the zinc-stabi-

lized samples was then accomplished according to directions previously described.

## DIRECT DETERMINATION OF MOLECULAR $H_2S$ IN AQUEOUS SOLUTION

The apparatus used for determination of molecular $H_2S$ is shown in Figure 2. The flow rate of compressed nitrogen from a cylinder is maintained by means of a two-stage gas regulator and a flow meter. The nitrogen is sparged through an approximate 16-in high column of test solution in a bubbler immersed in a 20-liter Pyrex glass carboy. The bubbler, described by Schneider and Freund,[42] was designed so that the rising bubbles cause circulation of the test solution in the container and prevent significant local depletion of sulfide. A medium porosity sintered glass disc (30 mm diameter) produces the desired bubble size.

A spray trap is inserted between the bubbler and the concentration column to ensure that no droplets of test solution are carried over in the nitrogen and deposited on the concentration column.

The concentration column is a 26-cm section of 10-mm diameter borosilicate glass tubing to which a three-way teflon buret stopcock has been fused and which is packed with glass beads of 3-mm diameter. The acutal concentration section of the column containing the beads is 18 cm in length. To facilitate installation and removal of the concentration column, 12/5 O-ring joints were fused to each end. The capillary tip of the three-way teflon buret stopcock facilitates complete delivery of the column washings into a 25-ml volumetric flask.

A 10-liter water displacement bottle is used to determine the volume of nitrogen dispersed in the solution and passed through the concentration column. The bottle is inverted and mounted on a supporting frame. A graduated glass tube of 8-mm diameter is inserted in a 3/4-inch hole drilled in the bottom of the bottle. The glass tube extends to within about 2 mm of the rubber stopper inserted in the neck of the

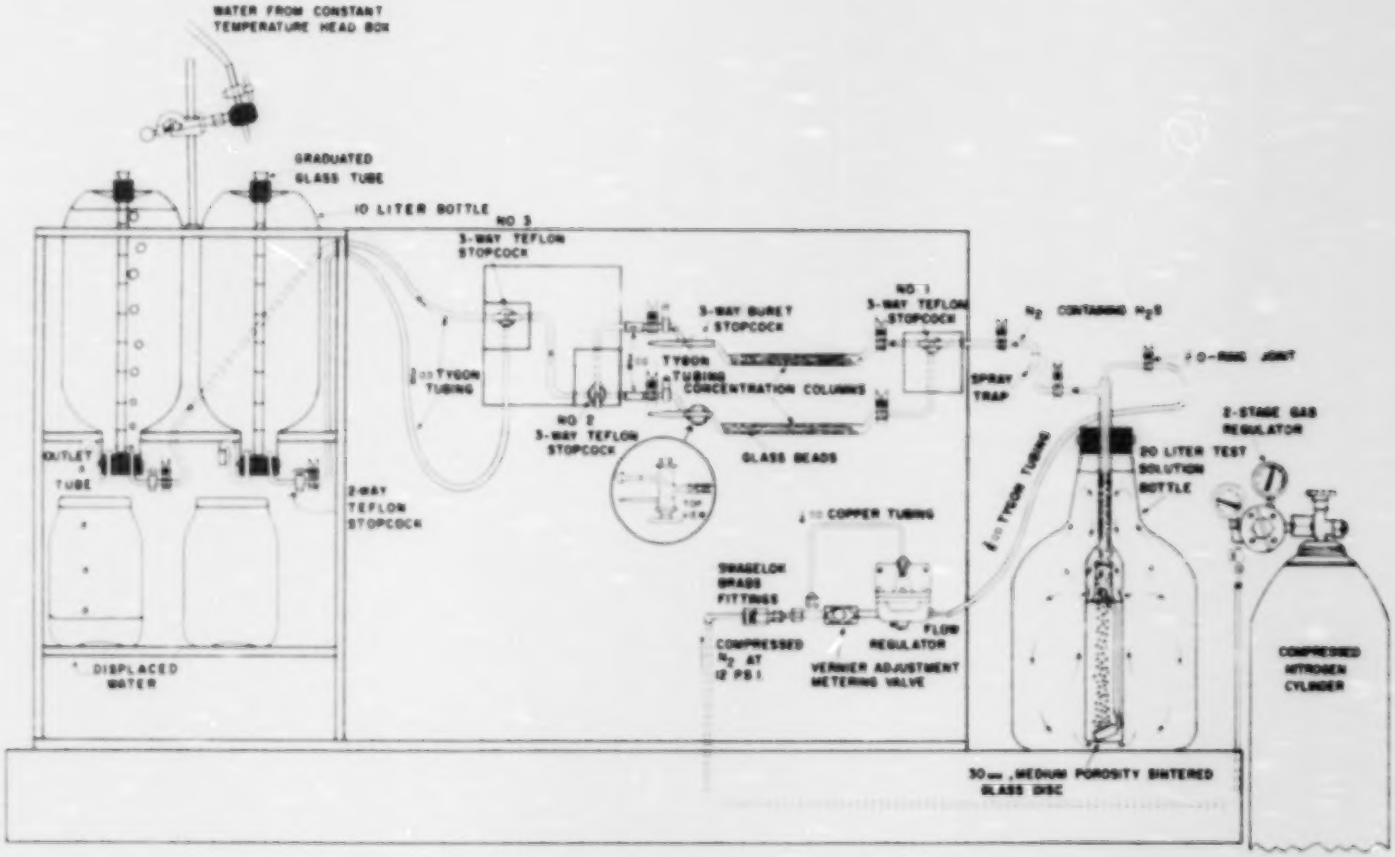


Figure 2. Apparatus used for distribution between water and nitrogen and concentration of molecular hydrogen sulfide. Insert shows top view of buret stopcock end of the concentration column.

bottle. An inlet tube for nitrogen and an outlet tube for displaced water are inserted into the stopper closing the neck of the bottle. A two-way teflon stopcock controls the flow of nitrogen into the bottle. The bottle need not be removed from the support except for cleaning purposes, because it can be filled through the 3/4-inch hole after each run.

With proper manipulation of the various stopcocks, the dispersed nitrogen from the bubbler can be passed through the concentration column and then to the water displacement bottle. By manipulation of three stopcocks, the nitrogen flow can be diverted from one concentration column to another, permitting continuous $H_2S$ determinations.

The solution whose molecular $H_2S$ content is to be determined is placed in a 20-liter carboy and the circulating glass bubbler is immersed in the solution. The bubbler is connected to the spray trap, which is connected to the No. 1 three-way teflon stopcock; all connections are made by means of Buna-N 12/5 O-ring joints. The regulator on the nitrogen cylinder is opened and its pressure adjusted to 12 lb/square inch. The needle valve on the flow meter is then adjusted so the compressed nitrogen is bubbled through the solution at a rate of usually between 25 and 50 ml/min. The No. 1 three-way teflon stopcock is positioned so that the displaced $H_2S$ will not pass through the arm of the stopcock to which the concentration column is to be connected, but instead will escape through the third arm, which at that time is open to the atmosphere. Compressed nitrogen is then bubbled through the test solution for 30 min to ensure equilibrium in the system before collection of $H_2S$ is begun.

The concentration column is prepared by coating the glass beads with 0.1 M zinc acetate. This coating is applied with the concentration column held in a vertical position, with the stopcock end down. A separatory funnel, containing 0.1 M zinc acetate, is connected to the buret stopcock at the lower end of the column by means of tygon tubing and an

O-ring joint. By raising and lowering the separatory funnel, the column can be filled and drained. When this procedure has been repeated four times, the O-ring joint is disconnected and the excess zinc acetate is allowed to drip out of the column. By weighing the concentration column before and after coating, the volume of 0.1 M zinc acetate adhering to the glass beads and column wall was determined to be about 0.5 ml. Slight variations in the amount of zinc acetate remaining in the column were demonstrated to have no effect on intensity of color development when the methylene blue method is used for the determination of sulfide. The column is then placed in position between the No. 1 and No. 2 three-way stopcocks and the O-ring joint at each end of the column is secured with a metal clamp. The concentration column is connected in series with a water displacement bottle by rotating, in the appropriate manner, the three-way teflon buret stopcock and the No. 2 and No. 3 teflon stopcocks, and by opening the two-way teflon stopcock in the nitrogen inlet tube of the water displacement bottle. The No. 1 three-way teflon stopcock then is rotated so the $H_2S$ equilibrated nitrogen coming from the bubbler passes through the concentration column and then continues through the system to displace water from the displacement bottle. In the concentration column the $H_2S$ in the nitrogen reacts with the zinc acetate to from zinc sulfide.

At the end of the concentration period, the column is removed from its collecting position and sulfide is analyzed according to a procedure described previously. The $H_2S$ concentration in the tested solution is derived by reference to a standard curve relating µg of $H_2S$ displaced per liter of nitrogen dispersed with the known $H_2S$ concentration in the standard solutions. The standard $H_2S$ solutions were prepared by diluting a known amount of $Na_2S \cdot 9H_2O$ with deoxygenated deionized water and lowering the pH of the test solution with HCl to about 4 or 5, where essentially all the sulfide present is as molecular $H_2S$. Since the quantity of $H_2S$ collected on the column is directly related to the $H_2S$ concentration in the test solution, the latter concentration can be

determined by reference to a calibration curve defined during this study.

In making a molecular $H_2S$ determination, it is necessary to know precisely the amount of nitrogen that has passed through the test solution and concentration column. Since gas regulators do not always deliver precisely the desired, sustained flow, it was decided to measure the nitrogen volume by means of a water displacement bottle (Figure 2). The inverted bottle is filled through the 3/4-inch hole in its bottom with deionized water at room temperature. The graduated glass tube is then inserted into this 3/4-inch hole and the rubber cap is removed from the S-shaped outlet tube. The water in the graduated glass tube drops to the level of the discharge end of the outlet tube and the displaced water is discarded. The two-way stopcock on the inlet line now can be opened and the nitrogen passing through the concentration column is allowed to enter the displacement bottle. The displaced water flows out through the S-shaped outlet tube and is collected. At the end of the concentration period, the two-way inlet stopcock is closed and a rubber cap is placed over the outlet tube opening. The displaced water is measured and corresponds to the total uncorrected volume of nitrogen dispersed. Water is then introduced into the graduated tube until the water level in the tube rises to the water level in the 10-liter displacement bottle. The amount of water added by way of this tube is referred to here as the "crude correction volume." To obtain the "true correction volume," it is necessary to determine how much water would be required to fill the graduated tube to the level of water in the displacement bottle if the correction tube were sealed off at the bottom. This value is called the "tube correction" and will not exceed 10 ml. The true correction is equal to the crude correction minus the tube correction. When the level of water in the tube equals the level of water in the displacement bottle, the gas above the water must be at atmospheric pressure, because the correction tube is open to the atmosphere at the top. Therefore the total volume of nitrogen dispersed is equal to the total uncorrected volume of nitrogen dispersed minus the true correction volume.

## ACUTE SULFIDE BIOASSAYS

### Experimental Water

Experimental water used in all sulfide bioassays came from a laboratory well which draws water from the Jordon Sandstone stratum underlying the Minneapolis-St. Paul metropolitan area. Chemical analysis of the well water by the Minnesota Department of Health, Division of Environmental Health, and the National Water Quality Laboratory, Duluth, Minnesota is given in Table 2.

### Experimental Fish

Juvenile fathead minnows (Pimephales promelas Rafinesque) were used as test organisms to study the toxicity of solutions containing sulfide at various pH values. The fathead minnow was chosen as an experimental organism because it can be cultured and maintained in a laboratory as well as handled with ease, and because it has a wide distribution in various chemically diverse natural waters from acid bog lakes (Dymond and Scott[50]) to lakes of high pH (Rawson and Moore,[51] McCarraher and Thomas[52]).

The juvenile fathead minnows used in all the bioassays were cultured in the fisheries laboratory at the University of Minnesota, St. Paul. Our culture was originally started in January 1972 with fathead minnows from the U.S. Environmental Protection Agency's National Water Quality Laboratory in Duluth, Minnesota. It was hoped that by using an inbred laboratory cultured strain of fish a consistent sensitivity to sulfide would be observed for tests at different times with fish of different stocks and that possible effects of disease stress and/or treatment of wild fish stocks would be eliminated.

Fathead minnows used in all tests were cultured under a constant photoperiod in 30-liter glass aquaria receiving a continuous supply of laboratory well water at 25 C and with pH of approximately 7.9. Six lots of fish were tested at various times during a 16-week period. Fish in

Table 2. ANALYSIS OF LABORATORY WELL WATER[a/]

(milligrams/liter)

| Item | Concentration |
|---|---|
| Total alkalinity as $CaCO_3$ | 230 |
| Total hardness as $CaCO_3$ | 220 |
| Calcium as $CaCO_3$ | 140 |
| Magnesium as $CaCO_3$ | 70 |
| Iron | 0.02 |
| Chloride | <1 |
| Sulfate | <5 |
| Sulfide | 0.0 |
| Fluoride | 0.22 |
| Total phosphates | 0.03 |
| Sodium | 6 |
| Potassium | 2 |
| Copper | 0.0004 |
| Manganese | 0.0287 |
| Zinc | 0.0044 |
| Cobalt, nickel | < 0.0005 |
| Cadmium, mercury | < 0.0001 |
| Ammonia nitrogen | 0.20 |
| Organic nitrogen | 0.20 |

[a/] Water taken from well head after iron removal and before aeration and heating; pH 7.5.

separate bioassays ranged in age from approxiamtely 12 to 15 weeks, in mean total length from 27.7 to 34.1 mm, and in mean weight of survivors from 0.227 to 0.422 g.

One week prior to a bioassay fish were removed from rearing tanks and transferred to 20-liter aquaria. Well water at 20 C was introduced to each aquaria at the rate of 0.5 liter/min and throughout an acclimation period. The oxygen concentration during acclimation was maintained above 6 mg/liter. The fish were fed Oregon moist and Glencoe pelleted food twice daily until 1 day prior to exposure to sulfide.

Experimental Apparatus and Conditions

Acute toxicity bioassays were performed in three identical diluter units each including one control and four treatment chambers. The test chambers were constructed of double-strength window glass and General Electric RTV adhesive, measure 50 x 25 x 20 cm deep, and contained 20 liters of test solution. The cyclic water-delivery and toxicant systems were modified from that described by Brungs and Mount[53] and Mount and Warner,[54] respectively. Flow through each chamber was at the rate of approximately 500 ml/min, affording 90% replacement in about 90 minutes.

The pH of the test water was controlled by dispensing a sulfuric acid or sodium hydroxide solution with a "dipping bird" into the head reservoirs. The temperature of the test water was thermostatically controlled at 20 C by a hot water stainless steel heat-exchange coil in each head reservoir. The test water was aerated in the head reservoirs to maintain oxygen concentrations in the test chambers at near 7.5 mg/liter. Test chambers were illuminated for 12 hr each day with a 40-watt incandescent bulb 10 inches above each chamber.

Experimental Design and Procedure

The 96-hr bioassays conducted in this study were designed to determine the relationship between pH of test solutions and apparent $H_2S$ toxicity.

Between two and three acute tests were performed with fathead minnows at each of six pH values, ranging between 6.5 and 8.7. Each series of tests consisted of three acute bioassays conducted in three sets of experimental chambers at the same time and on juveniles from the same lot. The desired concentrations of $H_2S$ within each set of treatment chambers were arranged in an appropriate series so that various degrees of percentage mortality would be observed in the four treatments. The toxicant concentrations were randomly assigned to treatment chambers before each set of bioassays. Stock solutions of sodium sulfide were prepared with reagent grade $Na_2S \cdot 9H_2O$ crystals and deionized water. One pellet of reagent grade sodium hydroxide was added to each liter of stock solution to raise the pH, thus retarding evolution of $H_2S$ from the "dipping bird" reservoirs.

After the concentration and amount of stock sulfide necessary to give the desired concentration of dissolved sulfide in the test chambers for a given pH series were determined from a trial run without fish, the test chambers were flushed with well water. Three days before initiation of the bioassay 10 fish were randomly assorted into each of the 12 treatment and three control chambers. Sulfuric acid or sodium hydroxide was then slowly added to the head reservoirs to attain the desired pH. The fish were acclimated to the specified pH for at least 2 days before introduction of the sulfide.

At the beginning of the bioassay the sulfide concentrations were raised to the desired levels within a period of less than 1 hr. During each bioassay water temperature, dissolved oxygen, and pH in each test chamber were measured daily. Alkalinity was determined by potentiometric titration with a standard $H_2SO_4$ solution to the successive bicarbonate and carbonic acid equivalence points, identified by the inflection in the titration curve. Dissolved oxygen was measured with a Winkler standardized galvanic-type membrane electrode meter and pH with a Corning model 12 immersion-type glass electrode meter. Dissolved sulfide concentrations, which were determined to be essentially the same as total

sulfide concentration, were measured in treatment chambers at least twice daily. Water samples taken from the center of each test chamber were stabilized with zinc acetate and analyzed for sulfide by a colorimetric procedure previously described in section 228 B-4a (2) of Standard Methods (APHA[1]). The concentration of molecular $H_2S$ in each treatment chamber was calculated for each sulfide determination using the daily pH and temperature measurements, and the $K_1$ ($H_2S$) equilibrium constants derived during this study.

The number of mortalities in each test chamber was recorded at 24-hr intervals. Total lengths of dead and surviving fish were measured, and survivors were weighed. At no time during these bioassays was any mortality in control chambers observed. Estimates of the concentration of $H_2S$ most likely to cause 50 per cent mortality (LC50) after 96 hr of exposure were made in this study from lines fitted mathematically by the BMD03S probit analysis computer program to plots of percentage mortality against log $H_2S$ concentration (Dixon[55]). The 96-hr LC16 and LC84 values were also calculated from the probit analysis regression equations and 95% confidence intervals for LC50 values were computed according to formulas proposed by Litchfield and Wilcoxon.[56] $(Chi)^2$ tests were applied to each group of data to determine variability and acceptability.

# SECTION V

# RESULTS AND INTERPRETATIONS

## DETERMINATION OF SULFIDE IN AQUEOUS SOLUTION

### Iodometric Sulfide Determination

The salt $Na_2S \cdot 9H_2O$ has been widely used as a source of sulfide in the preparation of standard solutions. Because there may be situations where the sulfide concentration in stock solutions cannot be analytically determined, definition of the relationship between concentration based on a weight measurement and that determined quantitatively by titration may be useful. A number of stock sulfide solutions containing about 100 mg/liter as S (0.75 g $Na_2S \cdot 9H_2O$/liter) were prepared and the sulfide concentrations were determined by an iodate-iodide procedure with thiosulfate titration using starch to detect the end point. The values obtained were then compared with the calculated concentrations determined on a weight and volume basis. In general, the weighed $Na_2S \cdot 9H_2O$ standards were quite accurate as determined by iodometric titration. When 20 ml of the sulfide stock was added to approximately 100 ml of boiled and cooled deionized water, followed by the addition of concentrated HCl and 10 ml of standard iodine solution, the average percentage of sulfide determined compared to that calculated for 29 samples was $97.18 \pm 0.94$. When the concentrated HCl was added first to approximately 100 ml of boiled and cooled deionized water, followed by the addition of the standard iodine and then stock sulfide solutions, the average percentage of sulfide determined compared to that calculated for 29 samples was $98.17 \pm 0.59$. Therefore, when unstabilized stock sulfide solutions

are prepared on a weight and volume basis, the calculated sulfide concentrations should be about 2 to 3 per cent higher than the actual values. These data suggest that the best procedure for iodometric standardization of unstabilized sulfide is acidification of deionized water with concentrated HCl, followed by addition of the standard iodine solution and then the stock sulfide, and titration of excess iodine with thiosulfate. The volume of concentrated HCl added to the deionized water before addition of the iodine and stock sulfide is not critical and can vary from 2 to at least 10 drops with no change in titrant volume.

The stability of stock sulfide solutions prepared with $Na_2S \cdot 9H_2O$ was examined over a 190-hr period for solutions prepared with boiled and cooled deionized water (deoxygenated) and initially containing about 100 mg/liter as S. The solutions were prepared and stored under an atmosphere of nitrogen in 1-liter Pyrex glass volumetric flasks and either exposed to laboratory light, kept in the dark, made alkaline to 0.1 N NaOH, or stabilized with zinc acetate to 0.025 M Zn. Iodometric analysis at various times after preparation on aliquots of the stock solutions indicated that storage in light or dark had no effect on the rate of degradation. The decrease in sulfide concentration occurred at a rate of about 0.025 mg/liter per hour as S following preparation. Addition of base to a final concentration of 0.1 N NaOH essentially doubled the rate of sulfide degradation to about 0.048 mg/liter per hour as S following preparation. The addition of zinc acetate had no effect on the iodometric titration when a 20-ml aliquot of sulfide solution in 100 ml of deionized water was acidified before addition of iodine. The solution is turbid white before addition of acid but the end point is the same as for similar sulfide solutions in which no zinc was present. If acid is added following the iodine, the volume of thiosulfate required to titrate the remaining iodine is somewhat less than when the acid is added before the iodine. The addition of zinc acetate to the stock sulfide solution to a concentration of 0.025 M Zn stabilized the sulfide

so that over a 190-hr period essentially no decrease in sulfide concentration could be detected.

Colorimetric Sulfide Determination

The stabilization of sulfide solutions with certain metal salts does not appear to interfere with sulfide determination by the colorimetric method utilized during this investigation and may even under some circumstances enhance the intensity of color by preventing sulfide degradation before color development can be accomplished. When 0.5 ml of a zinc acetate solution (0.1 M Zn), zinc chloride plus gelatin (0.1 M Zn), or cadmium hydroxide suspension (0.02 M Cd) were added to a known amount of sulfide, the amount of sulfide determined was essentially the same as that calculated to be present. However, when 0.5 ml of a 0.1 M cadmium sulfate or cadmium chloride solution was added to a known amount of sulfide, the amount of sulfide determined was about 4 and 8 per cent less, respectively, than the calculated concentration. It appears that under the conditions of color development employed during this study, these latter cadmium salts bind sulfide to such an extent that not all of the sulfide is released during the color development phase.

The colorimetric diamine reaction for sulfide determination is known to be pH-dependent and different authors have proposed various sulfuric acid molar concentrations at which maximum color production is realized. The molar acidity values discussed below merely represent calculated values based on dilutions of reagents containing sulfuric acid. The values probably do not represent hydrogen ion concentration since other reagents such as the diammonium hydrogen phosphate will buffer the sulfuric acid.

An attempt was made to define the molar acidity of reaction and measurement that would maximize color development when the reaction volume is 6.5 ml and that of measurement is 25 ml. No attempt was made to determine these precise conditions but it was felt that in order to have a reproducible and sensitive sulfide method, color production should be

nearly optimal. When utilizing the N,N-dimethyl diamine reagent the optimum color development occurred with absorbance determined at 666 nm when reaction molar acidity was about 0.75 to 1.0 and the measurement molar acidity was 0.25 to 0.26. However, when the quantity of N,N-dimethyl diamine reagent was varied from 0.8 to 1.1 ml with the resulting reaction and measurement molar acidities varying from 0.89 to 1.22 and 0.23 to 0.32, respectively, the amount of color development produced was essentially unchanged. Since color development was also not markedly affected when the volume of diammonium hydrogen phosphate was kept between 1.25 and 1.75 ml, slight variations in the amount of reagents added do not appear to affect color development significantly.

Use of the N,ethyl-N-hydroxyethyl- or N,N-diethyl diamine reagents produced near optimum color development with absorbance determined at 668 or 672 nm when the molar acidity of reaction was about 0.75 or 0.25 and the molar acidity of measurement was 0.20 to 0.25 or 0.20, respectively. The minimum molar acidity of measurement necessary to produce a clear solution in a total volume of 25 ml following the addition of 1.5 ml of diammonium hydrogen phosphate is 0.18. According to Rees *et al.*,[32] the optimum color development when using the N,N-diethyl diamine reagent is realized at a reaction and measurement molar acidity of about 0.09 without the addition of the phosphate reagent. If the molar acidity of reaction was 0.25 and the molar acidity of measurement 0.20, the optimum amount of diammonium hydrogen phosphate necessary to produce maximum color development using the N,N-diethyl diamine reagent was determined in this study to be 1.5 ml. However, the color development is not markedly affected when the volume of phosphate is kept between 1.25 and 1.75 ml.

Sulfide calibration curve solutions were prepared by dilution with freshly boiled and cooled deionized water of a stock solution of known concentration determined iodometrically. Each curve was defined immediately after the preparation of a sulfide standard solution. Addition of 0.5 ml zinc acetate (0.1 M Zn) to each flask stabilized the sulfide.

The regression equations summarizing the calibration curves prepared with the various diamine reagents and under different calculated acidity conditions when the diamine and iron reagents are added separately or in a 9 to 1 mixture are presented in Table 3. In the regression analysis X refers to sulfide concentration expressed as µg of $H_2S$ in a total volume of 25 ml. All absorbance values (Y), which never exceeded 1.0 unit, were corrected for a reagent blank and were determined at 666 nm for N,N-dimethyl, 668 nm for N,ethyl-N-hydroxyethyl, and 672 nm for N,N-diethyl diamine reagents. If a known amount of sulfide was added to a concentration column where color development occurred, the resulting absorbance readings were essentially the same as those determined for similar sulfide solutions in which color development was in a 25-ml volumetric flask. The slope of an N,N-diethyl diamine reagent calibration curve determined from data published by Rees et al.[32] was calculated to be 0.07120. This value applies when the reaction and measurement molar acidity is 0.09, no diammonium hydrogen phosphate added, sulfide expressed as µg $H_2S$ in a total volume of 25 ml, and absorbance determined for a 10 mm light path. When compared with the optimum slope of 0.06081 (Table 3) determined during the present study with N,N-diethyl diamine reagent, Rees' method is 1.17 times more sensitive to sulfide. However, this increase in sensitivity can only be attained by omitting the diammonium hydrogen phosphate, which may not be advisable in some instances, and by color development at reduced acidity.

When the various diamine reagents and iron chloride are added to the standard solutions separately, the color development is essentially unaffected by the age of the reagents over a period of at least 1 or 2 months. However, when the various diamine reagents and iron chloride solutions are added as a 9 to 1 mixture, color development in a standard sulfide solution is markedly affected by the age of the mixed reagent. With the N,N-dimethyl diamine reagent, used when the reaction molar acidity was 1.0 and measurement molar acidity 0.26, a given mixture will produce maximum color development for a period less than 30 min after preparation. With the N,ethyl-N-hydroxyethyl and N,N-diethyl

Table 3. LINEAR REGRESSION ANALYSIS OF CALIBRATION CURVES RELATING ABSORBANCE (Y) AND SULFIDE CONCENTRATION (X) IN μG $H_2S$ PER 25-ML FOR SOLUTIONS PREPARED WITH VARIOUS DIAMINE REAGENTS AND UNDER DIFFERENT ACIDITY CONDITIONS

| Diamine and iron reagents added | Molar acidity: Reaction | Molar acidity: Measurement | Calibration curves in regression | Linear regression analysis[a] (Y = A + BX): A | B | r | Sy·x[b] |
|---|---|---|---|---|---|---|---|
| **N,N-dimethyl-p-phenylene diamine oxalate** | | | | | | | |
| Separately | 1.0 | 0.26 | 8 | 0.0094 | 0.04724 | 0.9992 | 0.0085 |
| Mixture | 1.0 | 0.26 | 12 | 0.0109 | 0.04117 | 0.9990 | 0.0130 |
| **N,ethyl-N-hydroxyethyl-p-phenylene diamine sulfate** | | | | | | | |
| Separately | 0.75 | 0.20 | 4 | 0.0080 | 0.05908 | 0.9996 | 0.0070 |
| Separately | 1.0 | 0.26 | 8 | 0.0062 | 0.05880 | 0.9998 | 0.0053 |
| Mixture | 1.0 | 0.26 | 2 | 0.0102 | 0.06756 | 0.9997 | 0.0094 |
| **N,N-diethyl-p-phenylene diamine oxalate** | | | | | | | |
| Separately | 0.25 | 0.20 | 2 | 0.0122 | 0.06081 | 0.9990 | 0.0125 |
| Separately | 0.75 | 0.20 | 8 | 0.0119 | 0.05816 | 0.9989 | 0.0120 |
| Mixture | 1.0 | 0.26 | 2 | 0.0207 | 0.05594 | 0.9989 | 0.0154 |

[a] Reagent blank corrected absorbance values of less than 1.0 and for a light path of 10 mm were determined at 666, 668, and 672 nm when calibration curves were prepared with the N,N-dimethyl, N,ethyl-N-hydroxyethyl, and N,N-diethyl-p-phenylene diamine reagents, respectively.
[b] Standard error of the estimate or deviation of Y for fixed X.

diamine reagents, used when the reaction molar acidities are 0.75 and measurement molar acidities are 0.20, the mixtures will produce maximum color development for at least 4 hr after preparation. With the N,N-diethyl diamine reagent, used when the reaction molar acidity is 0.25 and measurement molar acidity is 0.20, the mixture will produce maximum color development for only a few minutes after preparation. Therefore, it appears that for reproducible and maximum color development sulfide solutions should be stabilized with zinc and the diamine and iron reagents should be added separately. An exception to this generalization would be with use of the N,ethyl-N-hydroxyethyl diamine reagent. In this case the intensity of color development was greater at comparable acidity levels when the reagents were added in a mixture than when added separately.

Concentration Column Sulfide Absorbants

The efficiency of $H_2S$ collection was studied by using two zinc acetate coated glass bead concentration columns placed in series with aliquots of standard sodium sulfide contained in the total sulfide reaction flask. The $H_2S$ uptake by each column was measured after 60 min of nitrogen stripping at 270 ml/min and the percentage efficiency of capture calculated. Since no detectable amount of sulfide was collected in the second column, it is assumed that all of the $H_2S$ reaching the first column is absorbed. There was no effect on amount of sulfide displacement and collection efficiency when the time of displacement varied between 60 and 180 min, however 30 min was inadequate. The recovery of a known amount of sulfide added to a total sulfide reaction flask by the first concentration column when coated with different metal salts was demonstrated to be reproducible but incomplete. The results summarizing the degree of $H_2S$ recovery by various metal salts, as indicated by the percentage of sulfide collected compared to that added to a reaction flask, are presented in Table 4. The sulfide absorbants prepared with zinc salts resulted in the highest recovery of sulfide when compared with those containing cadmium. The less than 100 per cent

Table 4. RECOVERY AND STABILIZATION OF $H_2S$ BY GLASS BEAD CONCENTRATION COLUMNS COATED WITH VARIOUS METAL SALTS

| Sulfide absorbant | Number of tests | Range in sulfide concentration,[a] µg $H_2S$ | Percentage sulfide recovered | |
|---|---|---|---|---|
| | | | Mean | SD |
| Zinc acetate (0.1 M Zn) | 19 | 3.00-15.68 | 98.3 | 3.7 |
| Zinc chloride plus gelatin (0.1 M Zn) | 5 | 5.18-10.80 | 97.4 | 3.7 |
| Cadmium hydroxide suspension (0.02 M Cd) | 4 | 10.31-10.58 | 93.2 | 2.2 |
| Cadmium chloride (0.1 M Cd) | 4 | 10.61-11.83 | 95.3 | 2.1 |
| Cadmium sulfate (0.1 M Cd) | 7 | 5.28-16.17 | 86.8 | 2.6 |

[a] Known amounts of sulfide were added to a total sulfide reaction flask.

sulfide recovery may have resulted from loss due to oxidation, adhesion to glass walls between the reaction flask and concentrations column, and transition to sulfides insoluble in acid due to impurities in reagents or deionized water. The stability of sulfide collected in a zinc acetate coated concentration column was determined by storing the columns in the dark or exposing them to laboratory fluorescent light for periods of 2 to 6 hr. No decline in sulfide recovery with storage time was observed.

Previous experiments have indicated that some cadmium salts may combine with sulfide and result in a non-quantitative release of sulfide during the methylene blue colorimetric test and consequent reduction in sulfide determined in comparison with quantities known to be present. Therefore, the reduction in percentage of sulfide collected from the amount added to the total sulfide reaction flask may be due in part to decreased efficiency in sulfide collection but is more than likely due to interference in the colorimetric test for determination of the cadmium stabilized sulfide.

The stability of various metal sulfides was determined by passing air over sulfide collected in a concentration column and comparing the percentage of sulfide collected following exposure to various amounts of oxygen to that added and displaced by nitrogen from a total sulfide reaction flask (Table 5). When compared with the data in Table 4, it is apparent that none of the metal stabilized sulfides are affected by exposure to relatively large amounts of air. Because nearly 100 per cent recovery and complete stabilization of displaced $H_2S$ could be attained by the zinc acetate sulfide absorbant, it was used as the metal salt for coating of concentration columns in subsequent experiments, the results of which are presented below.

Known amounts of sulfide were added to total sulfide reaction flasks, displaced with nitrogen and collected on zinc acetate coated concentration columns. These columns were then connected to carboys containing

Table 5. STABILITY OF METAL SULFIDES ON CONCENTRATION COLUMNS TO OXIDATION BY AIR

| Air flow, ml/min for 60 min | Oxygen,[a] moles | $H_2S$ added to reaction flask, µg | $H_2S$ collected on column, µg | Sulfide collected after exposure to air, % |
|---|---|---|---|---|
| | | Zinc acetate (0.1 M Zn) | | |
| 100 | 0.054 | 10.43 | 10.28 | 98.6 |
| 200 | 0.107 | 10.39 | 10.30 | 99.1 |
| 400 | 0.214 | 10.43 | 9.91 | 95.0 |
| | | Zinc chloride plus gelatin (0.1 M Zn) | | |
| 100 | 0.054 | 10.57 | 10.54 | 99.7 |
| 200 | 0.107 | 10.54 | 10.03 | 95.2 |
| 400 | 0.214 | 10.52 | 10.35 | 98.4 |
| | | Cadmium hydroxide suspension (0.02 M Cd) | | |
| 100 | 0.054 | 10.39 | 9.40 | 90.5 |
| 200 | 0.107 | 10.35 | 9.84 | 95.1 |
| 400 | 0.214 | 10.31 | 9.77 | 94.8 |
| | | Cadmium chloride (0.1 M Cd) | | |
| 100 | 0.054 | 10.65 | 10.37 | 97.4 |
| 200 | 0.107 | 10.61 | 10.23 | 96.4 |
| 400 | 0.214 | 10.63 | 10.06 | 94.6 |
| | | Cadmium sulfate (0.1 M Cd) | | |
| 100 | 0.054 | 10.77 | 9.62 | 89.3 |
| 200 | 0.107 | 10.77 | 9.35 | 86.8 |
| 400 | 0.214 | 10.73 | 9.01 | 84.0 |

[a] Assume air is 20% $O_2$ and 1 mole $O_2$ occupies 22.4 liters.

20 liters of aerated well water and nitrogen was passed over the columns after being dispersed through the water at approximately 140 ml/min for 4 to 5 hr. The initial and final dissolved oxygen concentrations in the carboys were about 10 and 0.3 mg/liter, respectively. The oxygen displaced from the well water and passed over the sulfide collected on the concentration columns had no effect on the stability of the sulfide collected since essentially 100 per cent of the sulfide added to the total sulfide reaction flasks was subsequently determined on the concentration columns. The maximum amount of dissolved oxygen displaced from 20 liters of water containing 10 mg/liter $O_2$ is 0.00625 mole. This is considerably less than the amount of oxygen demonstrated (Table 5) to have no adverse effect on zinc stabilized sulfide. Therefore, oxidation of zinc stabilized sulfide on a concentration column by oxygen displaced from test solutions would be negligible.

Because the recovery of sulfide by the indirect total sulfide method is incomplete though reproducible, it was necessary to prepare a calibration curve from standards varying in volume from 50 to 200 ml and containing $H_2S$ from 2.71 to 11.66 µg following the same procedure as for the samples. The standards were prepared one at a time and carried through the sulfide evolution and collection procedure. The relationship between µg $H_2S$ (X) added to the reaction flask and that collected (Y) on a zinc acetate coated concentration column was determined from 18 standard solutions and can be defined with $r = 0.994$ by the regression equation:

$$Y = -0.156 + 0.984\ X. \qquad (10)$$

The overall average percentage of $H_2S$ collected compared with that added was $95.7 \pm 4.7$ per cent. From the above equation the µg of sulfide expressed as $H_2S$ in a sample of known volume can be calculated from the µg of $H_2S$ collected in a concentration column following recovery by the indirect total sulfide method. If a sample is stabilized with zinc

acetate, analysis for total sulfide by the indirect method can be made without any decrease in percentage recovery with samples stored for at least [illegible] hr. However, sulfide stabilization should occur in a reaction flask because when performed in a volumetric flask and then transferred to the reaction flask, a lower percentage recovery was observed.

## DIRECT DETERMINATION OF MOLECULAR $H_2S$ IN AQUEOUS SOLUTION

The precision with which the vapor phase equilibration method can be used for the direct determination of molecular $H_2S$ depends in large part on the effect which the height of the nitrogen bubbling column and the rate of nitrogen dispersion have on the µg of $H_2S$ displaced per liter of nitrogen dispersed. Experiments conducted with six different bubbling depths demonstrated that there is only a slight effect on $H_2S$ displacement rate when the water column in a bubbler varies in height from 13 to 95 cm. During any single determination of molecular $H_2S$ the maximum variation in column height ranged from about 35 to 50 cm. Therefore, at no time did the rate of $H_2S$ displacement have to be corrected for bubbling column height. It was also demonstrated that bubble size was not critical since no measurable difference in displacement rate of $H_2S$ was observed when the nitrogen was dispersed through a medium or a coarse gas dispersion frit. When the nitrogen dispersion rate ranged from 25 to 200 ml/min, the µg of $H_2S$ displaced per liter of nitrogen dispersed was the same for solutions containing essentially equal $H_2S$ concentrations. Therefore, as long as the total volume of nitrogen dispersed through a test solution is known, no correction has to be made for the rate of dispersion over the tested range.

The rate at which a gas in solution can be displaced is a function of the solution's temperature. The rate at which $H_2S$ is displaced per liter of nitrogen dispersed was determined for a number of test solutions containing different $H_2S$ concentrations ranging from about 5 to 200 µg/liter and at temperatures of 10, 15, 20, or 25 C. A summary of these data is presented in Table 6 where partition coefficients relating

Table 6. $H_2S$ DISPLACEMENT BY NITROGEN DISPERSED THROUGH TEST SOLUTIONS OF KNOWN MOLECULAR $H_2S$ CONCENTRATION AND TEMPERATURE

| Temperature, C | Determined $H_2S$ in solution, [a] µg/l | $H_2S$ displaced/ 1 $N_2$ dispersed, µg | Partition coefficient [b] µg $H_2S$ displaced/1 $N_2$ µg/l $H_2S$ in solution | $\log_{10}$ |
|---|---|---|---|---|
| 10.0 | 5.039 | 1.243 | 0.2467 | -0.6078 |
| 10.0 | 10.75 | 2.852 | 0.2653 | -0.5763 |
| 10.0 | 25.88 | 6.959 | 0.2689 | -0.5704 |
| 10.2 | 50.86 | 13.61 | 0.2676* | -0.5725 |
| 10.3 | 80.68 | 20.34 | 0.2521 | -0.5984 |
| 10.1 | 145.6 | 37.66 | 0.2587 | -0.5872 |
| 10.0 | 215.7 | 56.33 | 0.2611 | -0.5832 |
| | | | Mean 0.2601 | |
| | | | SD 0.0082 | |
| | | | CV 3.15 | |
| 15.1 | 4.516 | 1.297 | 0.2872 | -0.5418 |
| 15.1 | 9.756 | 2.964 | 0.3041 | -0.5170 |
| 15.1 | 25.15 | 7.304 | 0.2904 | -0.5370 |
| 15.1 | 56.52 | 16.38 | 0.2898* | -0.5379 |
| 15.2 | 83.12 | 25.51 | 0.3069 | -0.5130 |
| 15.2 | 153.3 | 45.74 | 0.2984* | -0.5252 |
| 15.2 | 198.0 | 62.69 | 0.3166 | -0.4995 |
| | | | Mean 0.2991 | |
| | | | SD 0.0108 | |
| | | | CV 3.61 | |
| 20.0 | 4.592 | 1.613 | 0.3513 | -0.4543 |
| 20.0 | 9.600 | 3.208 | 0.3342 | -0.4760 |
| 20.0 | 24.83 | 8.315 | 0.3349 | -0.4751 |
| 20.0 | 46.30 | 15.13 | 0.3268 | -0.4857 |
| 20.0 | 69.36 | 23.10 | 0.3330 | -0.4776 |

Table 6 (continued). $H_2S$ DISPLACEMENT BY NITROGEN DISPERSED THROUGH TEST SOLUTIONS OF KNOWN MOLECULAR $H_2S$ CONCENTRATION AND TEMPERATURE

| Temperature, C | Determined $H_2S$ in solution, [a] µg/l | $H_2S$ displaced/ 1 $N_2$ dispersed, µg | | Partition coefficient [b] µg $H_2S$ displaced/1 $N_2$ µg/l $H_2S$ in solution | $\log_{10}$ |
|---|---|---|---|---|---|
| 20.0 | 127.0 | 46.21 | | 0.3639 | -0.4390 |
| 19.9 | 218.1 | 83.15 | | 0.3812 | -0.4188 |
| | | | Mean | 0.3465 | |
| | | | SD | 0.0199 | |
| | | | CV | 5.74 | |
| 24.9 | 5.143 | 2.079 | | 0.4042 | -0.3934 |
| 25.0 | 8.861 | 3.492 | | 0.3941 | -0.4044 |
| 24.9 | 25.57 | 10.50 | | 0.4106 | -0.3866 |
| 24.9 | 42.45 | 17.93 | | 0.4224* | -0.3743 |
| 24.9 | 47.51 | 18.57 | | 0.3909 | -0.4079 |
| 24.9 | 82.15 | 33.49 | | 0.4077 | -0.3896 |
| 24.9 | 155.5 | 62.67 | | 0.4030 | -0.3947 |
| 25.0 | 191.7 | 78.33 | | 0.4086 | -0.3887 |
| | | | Mean | 0.4052 | |
| | | | SD | 0.0098 | |
| | | | CV | 2.42 | |

[a] pH values of sulfide test solutions were between 4 and 5.
[b] Partition coefficients marked with an asterisk correspond to solutions prepared with deoxygenated well water; all others prepared with deoxygenated deionized water; SD - standard deviation; CV - coefficient of variability (%).

the µg $H_2S$ displaced per liter nitrogen dispersed to the known µg/liter $H_2S$ concentration in the test solution are calculated. The partition coefficients (Y) were log linear with respect to temperature (X) and a regression equation defining this relationship with r = 0.9771 is:

$$\log Y = -0.7188 + 0.01301 X. \quad (11)$$

This relationship was subsequently used to calculate the concentration of $H_2S$ in a test solution when temperature and µg of $H_2S$ displaced per liter nitrogen dispersed through the solution were known. The rate at which $H_2S$ is displaced from solution is independent of whether the solutions are prepared with deionized or well water, so the above regression equation is applicable to all test solutions prepared during this study.

EQUILIBRIUM CONSTANTS FOR THE FIRST DISSOCIATION OF $H_2S_{(aq)}$

Assuming the concentration of the $S^{2-}$ ion to be negligible in solutions prepared during this study, the relationship between dissolved sulfide species and pH for a test solution at particular temperatures can be defined from the first dissociation constant of $H_2S_{(aq)}$ (equation no. 2). Therefore, the apparent first dissociation constants and $pK_1$ values of $H_2S_{(aq)}$ were determined at 10, 15, 20, and 25 C. The test solutions used contained different total sulfide concentrations, ranging from about 25 to 2,800 µg/liter as $H_2S$, prepared with either deoxygenated deionized water or well water and having various pH values ranging from about 6.1 to 8.7. The pH of these solutions was controlled by the addition of 200 ml of the appropriate 1/15 M phosphate buffer and small amounts of weak $H_2SO_4$ or NaOH to a total volume of 20 liters. Measurements of temperature, pH, total sulfide, and $H_2S$ displacement rate were made on each test solution. From the relationship between the partition coefficients and temperature, previously described in equation no. 11, the molecular $H_2S$ concentration in each test solution could be calculated. A summary of these results is presented in Table 7.

Linear regression analyses of the $K_1$ dissociation constants and $pK_1$ values in Table 7 for temperatures ranging from 10 to 25 C are presented in Table 8. The slight difference in the linear regression equation for solutions prepared either with deionized or well water may be due to differences in test solution ionic strength or from the presence of minute amounts of total sulfide occurring as metal sulfides in test solutions prepared with well water. The well water test solutions represent natural waters of fairly high alkalinity and hardness. Therefore, the linear regression equation for the combined data from deionized and well water test solutions is proposed for defining the relationship between the apparent first dissociation constant of $H_2S_{(aq)}$ and temperature and would be applicable to most freshwaters of low ionic strength. The relationship between $pK_1$ and temperature (T) in degrees Celcius (C) gives the best fit by linear regression analysis, therefore the equation

$$pK_1 = 7.252 - 0.01342\ T\ (C) \qquad (12)$$

was used to define the first dissociation constant of $H_2S_{(aq)}$ at various temperatures in subsequent calculations. Data used in calculating this equation were obtained from solutions prepared over the temperature range of 10 to 25 C. However, because of the "good fit" of these data to the linear regression equation, it was felt that extrapolation to other temperatures, as justified from the relationship presented in Figure 1, would permit acceptable predictions of $pK_1$ at temperatures ranging from at least 5 to 30 C.

By calculation it can be demonstrated that the relationship between $[H_2S]/[\text{dissolved sulfide}]$ is equal to the factor $[H^+]/K_1 + [H^+]$, assuming $[S^{2-}]$ to be negligible. When both the molecular $H_2S$ and dissolved sulfide concentrations are expressed as $H_2S$ and in the same units (i.e., µg/liter $H_2S$), dissolved sulfide as µg/liter $H_2S$ times the factor will equal molecular $H_2S$ in µg/liter. Factors presented in Table 9 define the fraction of dissolved sulfide as molecular $H_2S$ at various temperatures from 5 to 30 C in 1.0 degree intervals and pH values from

Table 7. APPARENT $K_1$ DISSOCIATION CONSTANTS AND $pK_1$ VALUES OF $H_2S(aq)$ DETERMINED FOR TEST SOLUTIONS OF DIFFERENT TEMPERATURES, pH VALUES, AND TOTAL SULFIDE CONCENTRATIONS

| Temperature, C | pH | Determined total sulfide, µg/l as $H_2S$ | $H_2S$ displaced/liter $N_2$ dispersed, µg | Partition coefficient[a] | Determined $H_2S$, µg/l | Equilibrium constants[b] $K_1 \cdot 10^7$ | $pK_1$ |
|---|---|---|---|---|---|---|---|
| | | | | Deionized water | | | |
| 10.0 | 6.235 | 25.52 | 5.834 | 0.2578 | 22.63 | 0.7422 | 7.129 |
| 10.0 | 6.270 | 85.55 | 19.30 | 0.2578 | 74.85 | 0.7681 | 7.115 |
| 10.0 | 7.066 | 47.96 | 6.806 | 0.2578 | 26.40 | 0.7014 | 7.154 |
| 10.1 | 7.021 | 156.9 | 22.23 | 0.2586 | 85.98 | 0.7861 | 7.104 |
| 10.2 | 7.678 | 168.0 | 9.626 | 0.2594 | 37.11 | 0.7406 | 7.130 |
| 10.2 | 7.674 | 598.3 | 33.92 | 0.2594 | 130.8 | 0.7574 | 7.121 |
| 10.0 | 8.497 | 1123 | 11.07 | 0.2578 | 42.95 | 0.8009 | 7.096 |
| | | | | Well water | | | |
| 10.1 | 7.509 | 546.7 | 41.19 | 0.2586 | 159.3 | 0.7533 | 7.123 |
| 10.1 | 7.674 | 566.4 | 33.22 | 0.2586 | 128.4 | 0.7222 | 7.141 |
| 10.0 | 8.581 | 1370 | 11.74 | 0.2578 | 45.53 | 0.7634 | 7.117 |
| 9.9 | 8.516 | 2329 | 20.12 | 0.2571 | 78.28 | 0.8764 | 7.057 |
| | | | | | Mean | 0.7647 | 7.117 |
| | | | | | SD | 0.0462 | 0.0255 |
| | | | | | CV | 6.04 | 0.359 |

Table 7 (continued). APPARENT $K_1$ DISSOCIATION CONSTANTS AND $pK_1$ VALUES OF $H_2S_{(aq)}$

| Temperature, C | pH | Determined total sulfide, µg/l as $H_2S$ | $H_2S$ displaced/liter $N_2$ dispersed, µg | Partition coefficient[a] | Determined $H_2S$, µg/l | Equilibrium constants[b] $K_1 \cdot 10^7$ | $pK_1$ |
|---|---|---|---|---|---|---|---|
| | | | | Deionized water | | | |
| 15.0 | 6.178 | 31.14 | 8.182 | 0.2995 | 27.32 | 0.9289 | 7.032 |
| 14.9 | 6.157 | 83.09 | 22.34 | 0.2986 | 74.81 | 0.7715 | 7.113 |
| 15.0 | 7.095 | 57.80 | 8.463 | 0.2995 | 28.26 | 0.8400 | 7.076 |
| 15.0 | 7.044 | 141.9 | 21.17 | 0.2995 | 70.69 | 0.9100 | 7.041 |
| 14.9 | 7.714 | 155.7 | 8.205 | 0.2986 | 27.48 | 0.9012 | 7.045 |
| 15.0 | 7.653 | 570.9 | 33.53 | 0.2995 | 112.0 | 0.9114 | 7.040 |
| 14.9 | 8.473 | 1031 | 10.44 | 0.2986 | 34.98 | 0.9582 | 7.019 |
| | | | | Well water | | | |
| 15.2 | 7.553 | 489.5 | 35.01 | 0.3013 | 116.2 | 0.8993 | 7.046 |
| 15.1 | 7.673 | 578.9 | 33.81 | 0.3004 | 112.6 | 0.8797 | 7.056 |
| 15.0 | 8.520 | 1198 | 11.38 | 0.2995 | 38.01 | 0.9215 | 7.036 |
| 14.9 | 8.657 | 2803 | 19.57 | 0.2986 | 65.54 | 0.9200 | 7.036 |
| | | | | | Mean | 0.8947 | 7.049 |
| | | | | | SD | 0.0505 | 0.0256 |
| | | | | | CV | 5.65 | 0.364 |

Table 7 (continued). APPARENT $K_1$ DISSOCIATION CONSTANTS AND $pK_1$ VALUES OF $H_2S_{(aq)}$

| Temperature, C | pH | Determined total sulfide, µg/l as $H_2S$ | $H_2S$ displaced/liter $N_2$ dispersed, µg | Partition coefficient[a] | Determined $H_2S$, µg/l | Equilibrium constants[b] $K_1 \cdot 10^7$ | $pK_1$ |
|---|---|---|---|---|---|---|---|
| | | | | Deionized water | | | |
| 20.0 | 6.116 | 26.07 | 7.985 | 0.3479 | 22.95 | 1.041 | 6.983 |
| 19.9 | 6.400 | 76.42 | 21.96 | 0.3468 | 63.31 | 1.037 | 6.984 |
| 20.0 | 7.076 | 56.51 | 8.633 | 0.3479 | 24.81 | 1.072 | 6.970 |
| 19.9 | 7.056 | 174.2 | 27.74 | 0.3468 | 79.99 | 1.035 | 6.985 |
| 20.3 | 7.696 | 192.8 | 11.12 | 0.3510 | 31.68 | 1.024 | 6.990 |
| 20.1 | 7.734 | 839.0 | 46.31 | 0.3489 | 132.7 | 0.9816 | 7.008 |
| 20.0 | 8.499 | 1163 | 12.89 | 0.3479 | 37.06 | 0.9628 | 7.016 |
| | | | | Well water | | | |
| 20.0 | 7.576 | 367.6 | 25.81 | 0.3479 | 74.18 | 1.050 | 6.979 |
| 20.3 | 7.714 | 728.7 | 40.33 | 0.3510 | 114.9 | 1.032 | 6.986 |
| 19.9 | 8.515 | 1143 | 11.55 | 0.3468 | 33.30 | 1.018 | 6.992 |
| 19.9 | 8.582 | 2295 | 19.80 | 0.3468 | 57.11 | 1.026 | 6.989 |
| | | | | | Mean | 1.025 | 6.989 |
| | | | | | SD | 0.0303 | 0.0128 |
| | | | | | CV | 2.96 | 0.184 |

Table 7 (continued). APPARENT $K_1$ DISSOCIATION CONSTANTS AND $pK_1$ VALUES OF $H_2S_{(aq)}$

| Temperature, C | pH | Determined total sulfide, µg/l as $H_2S$ | $H_2S$ displaced/liter $N_2$ dispersed, µg | Partition coefficient[a/] | Determined $H_2S$, µg/l | Equilibrium constants[b/] $K_1 \cdot 10^7$ | $pK_1$ |
|---|---|---|---|---|---|---|---|
| | | | | Deionized water | | | |
| 25.1 | 6.307 | 31.81 | 10.40 | 0.4053 | 25.66 | 1.183 | 6.927 |
| 25.0 | 6.311 | 84.90 | 27.13 | 0.4041 | 67.14 | 1.293 | 6.889 |
| 25.0 | 7.059 | 54.28 | 8.944 | 0.4041 | 22.13 | 1.268 | 6.897 |
| 24.9 | 7.063 | 166.3 | 28.87 | 0.4029 | 71.66 | 1.142 | 6.942 |
| 25.0 | 7.688 | 176.5 | 10.07 | 0.4041 | 24.93 | 1.247 | 6.904 |
| 24.9 | 7.674 | 426.7 | 26.13 | 0.4029 | 64.86 | 1.182 | 6.927 |
| 25.0 | 8.472 | 1115 | 11.86 | 0.4041 | 29.35 | 1.247 | 6.904 |
| | | | | Well water | | | |
| 25.0 | 7.534 | 474.7 | 39.71 | 0.4041 | 98.26 | 1.120 | 6.951 |
| 25.0 | 7.557 | 497.1 | 38.58 | 0.4041 | 95.46 | 1.167 | 6.933 |
| 25.1 | 8.424 | 1179 | 13.22 | 0.4053 | 32.62 | 1.324 | 6.878 |
| 25.0 | 8.518 | 2256 | 21.47 | 0.4041 | 53.13 | 1.258 | 6.900 |
| | | | | | Mean | 1.221 | 6.914 |
| | | | | | SD | 0.0656 | 0.0234 |
| | | | | | CV | 5.37 | 0.338 |

a/ Partition coefficients calculated from the equation $\log Y = -0.7188 + 0.01301 X$ (equation no. 11).
b/ Calculated by assuming dissolved sulfide = $[H_2S] + [HS^-]$ and $K_1 = [H^+][HS^-]/[H_2S]$.

Table 8. RELATIONSHIP BETWEEN APPARENT $K_1$ DISSOCIATION CONSTANTS AND $pK_1$ VALUES OF $H_2S_{(aq)}$ FOR TEMPERATURES RANGING FROM 10 TO 25 C

| Test water | Linear regression analysis[a] (Y = A + BX) A | B | Correlation coefficient, r | Standard error of estimate, Sy·x |
|---|---|---|---|---|
| | | $K_1$ | | |
| Deionized | $0.4340 \cdot 10^{-7}$ | $0.03076 \cdot 10^{-7}$ | 0.9620 | 0.04862 |
| Well | $0.4780 \cdot 10^{-7}$ | $0.02882 \cdot 10^{-7}$ | 0.9495 | 0.05324 |
| Combined | $0.4500 \cdot 10^{-7}$ | $0.03005 \cdot 10^{-7}$ | 0.9569 | 0.05085 |
| | | $pK_1$ | | |
| Deionized | 7.261 | -0.01379 | -0.9644 | 0.02107 |
| Well | 7.238 | -0.01278 | -0.9550 | 0.02218 |
| Combined | 7.252 | -0.01342 | -0.9601 | 0.02180 |

[a] Y refers to $K_1$ or $pK_1$ values and X to temperature in degrees C.

6.0 to 9.0 in 0.1 unit intervals. These factors were calculated from equation 12.

A general acceptance by biologists of the apparently incorrect factors proposed by Pomeroy[2] prompted preparation of a table to be used to convert molecular $H_2S$ concentrations calculated from Pomeroy's factors to normalized concentrations corresponding to values obtained if the factors in Table 9 had been used originally. Table 10 defines this relationship between the fraction of dissolved sulfide as molecular $H_2S$ derived in this study (Table 9) and the fraction derived from Pomeroy's 1941 work corresponiding to a "typical water supply" at different pH values ranging from 6.0 to 9.0 and temperatures from 10 to 30 C. Molecular $H_2S$ concentrations have also in previous publications been calculated from constants

presented in Standard Methods from 1946 to 1965 (9th through 12th editions). These values can be converted to $H_2S$ concentrations based on data collected during this study by multiplying the reported concentration by the appropriate value in Table 11 at the corresponding pH. The factors presented in the 9th through 12th editions of Standard Methods of 0.29 at pH 7.1 and 0.23 at pH 7.3 do not correspond to the original values as found in Table IV of Pomeroy's 1941 work of 0.28 and 0.20, respectively. A similar correction can be made for $H_2S$ concentrations calculated from the factors presented in the 13th edition of Standard Methods (APHA[1]). Over the pH range of 6.0 to 8.8 and at 25 C the ratio of fractions calculated in this investigation to the reported values averaged 1.04 $\pm$ 0.02. Therefore, the fraction of dissolved sulfide as molecular $H_2S$ as calculated from these most recent factors at temperatures near 25 C would be very close to the values determined from factors derived in this study.

## $H_2S$ DETERMINATION IN VARIOUS WATERS AND EFFLUENTS

The feasibility of calculating molecular $H_2S$ concentrations in various waters, spiked with known amounts of sulfide, from the determined dissolved sulfide concentration and the fraction of dissolved sulfide as $H_2S$ under the experimental conditions was evaluated by comparing calculated values to direct determinations by the vapor phase equilibration technique. A summary of the tests performed in various types of waters and effluents is presented in Tables 12 and 13, respectively.

The vapor phase equilibration technique for the direct determination of molecular $H_2S$ is not strictly applicable to certain test solutions under static conditions since the degradation of sulfide occurs at too rapid a rate to estimate accurately initial $H_2S$ levels. However, by making repeated direct $H_2S$ determinations and interpolating, the molecular $H_2S$ concentration could be calculated at the time of sampling for total or dissolved sulfide. The oxygenated well water test solution did not show a rapid decline in total sulfide during the vapor phase equilibration

Table 9. FRACTION OF DISSOLVED SULFIDE AS MOLECULAR $H_2S$ IN AQUEOUS SULFIDE SOLUTIONS OF LOW IONIC STRENGTH[a/]

| | Temperature, C | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| 6.0 | .9387 | .9369 | .9351 | .9332 | .9312 | .9292 | .9272 | .9250 | .9229 | .9206 | .9184 | .9160 | .9136 |
| 6.1 | .9241 | .9219 | .9196 | .9173 | .9149 | .9125 | .9100 | .9074 | .9048 | .9021 | .8994 | .8965 | .8936 |
| 6.2 | .9063 | .9036 | .9009 | .8981 | .8952 | .8923 | .8893 | .8862 | .8830 | .8798 | .8765 | .8731 | .8697 |
| 6.3 | .8848 | .8816 | .8783 | .8750 | .8716 | .8681 | .8645 | .8608 | .8571 | .8533 | .8494 | .8454 | .8413 |
| 6.4 | .8591 | .8554 | .8515 | .8476 | .8435 | .8394 | .8352 | .8309 | .8265 | .8220 | .8175 | .8128 | .8081 |
| 6.5 | .8289 | .8245 | .8200 | .8154 | .8107 | .8059 | .8010 | .7960 | .7910 | .7858 | .7806 | .7752 | .7698 |
| 6.6 | .7938 | .7886 | .7835 | .7782 | .7728 | .7673 | .7618 | .7561 | .7504 | .7445 | .7386 | .7326 | .7265 |
| 6.7 | .7535 | .7477 | .7419 | .7359 | .7298 | .7237 | .7175 | .7112 | .7048 | .6983 | .6918 | .6852 | .6785 |
| 6.8 | .7083 | .7019 | .6954 | .6888 | .6821 | .6754 | .6686 | .6617 | .6548 | .6477 | .6407 | .6335 | .6263 |
| 6.9 | .6586 | .6516 | .6445 | .6374 | .6303 | .6230 | .6158 | .6084 | .6010 | .5936 | .5861 | .5786 | .5711 |
| 7.0 | .6051 | .5977 | .5902 | .5827 | .5752 | .5676 | .5600 | .5524 | .5448 | .5371 | .5294 | .5217 | .5140 |
| 7.1 | .5489 | .5413 | .5336 | .5259 | .5182 | .5105 | .5028 | .4950 | .4873 | .4796 | .4719 | .4642 | .4565 |
| 7.2 | .4915 | .4838 | .4761 | .4684 | .4607 | .4531 | .4454 | .4378 | .4302 | .4226 | .4151 | .4076 | .4002 |
| 7.3 | .4344 | .4268 | .4192 | .4117 | .4043 | .3969 | .3895 | .3822 | .3749 | .3677 | .3605 | .3534 | .3464 |
| 7.4 | .3789 | .3716 | .3644 | .3573 | .3502 | .3432 | .3363 | .3295 | .3227 | .3160 | .3093 | .3028 | .2963 |
| 7.5 | .3264 | .3196 | .3129 | .3063 | .2998 | .2934 | .2870 | .2807 | .2745 | .2684 | .2624 | .2565 | .2506 |

Table 9 (continued). FRACTION OF DISSOLVED SULFIDE AS MOLECULAR $H_2S$ IN AQUEOUS SULFIDE SOLUTIONS OF LOW IONIC STRENGTH[a/]

| | Temperature, C | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| 7.6 | .2779 | .2717 | .2657 | .2597 | .2538 | .2480 | .2423 | .2366 | .2311 | .2257 | .2203 | .2151 | .2099 |
| 7.7 | .2341 | .2286 | .2232 | .2179 | .2127 | .2076 | .2025 | .1976 | .1927 | .1880 | .1833 | .1787 | .1742 |
| 7.8 | .1954 | .1906 | .1859 | .1812 | .1767 | .1722 | .1679 | .1636 | .1594 | .1553 | .1513 | .1474 | .1435 |
| 7.9 | .1617 | .1576 | .1535 | .1495 | .1456 | .1418 | .1381 | .1345 | .1309 | .1274 | .1241 | .1207 | .1175 |
| 8.0 | .1329 | .1293 | .1259 | .1225 | .1193 | .1160 | .1129 | .1099 | .1069 | .1040 | .1011 | .09834 | .09564 |
| 8.1 | .1085 | .1055 | .1027 | .09985 | .09711 | .09443 | .09183 | .08928 | .08680 | .08438 | .08203 | .07973 | .07749 |
| 8.2 | .08815 | .08570 | .08331 | .08098 | .07871 | .07650 | .07434 | .07224 | .07020 | .06821 | .06627 | .06439 | .06255 |
| 8.3 | .07131 | .06929 | .06733 | .06541 | .06355 | .06174 | .05997 | .05825 | .05658 | .05495 | .05337 | .05183 | .05033 |
| 8.4 | .05749 | .05584 | .05423 | .05267 | .05115 | .04967 | .04823 | .04683 | .04547 | .04415 | .04286 | .04161 | .04040 |
| 8.5 | .04621 | .04487 | .04356 | .04229 | .04106 | .03986 | .03869 | .03756 | .03646 | .03539 | .03435 | .03334 | .03236 |
| 8.6 | .03706 | .03597 | .03492 | .03389 | .03289 | .03192 | .03098 | .03007 | .02918 | .02832 | .02748 | .02667 | .02588 |
| 8.7 | .02966 | .02879 | .02794 | .02711 | .02631 | .02553 | .02477 | .02403 | .02332 | .02263 | .02195 | .02130 | .02066 |
| 8.8 | .02371 | .02300 | .02232 | .02165 | .02101 | .02038 | .01978 | .01918 | .01861 | .01806 | .01752 | .01699 | .01648 |
| 8.9 | .01892 | .01836 | .01781 | .01728 | .01676 | .01626 | .01577 | .01530 | .01484 | .01440 | .01396 | .01354 | .01314 |
| 9.0 | .01509 | .01464 | .01420 | .01377 | .01336 | .01296 | .01257 | .01219 | .01182 | .01147 | .01112 | .01079 | .01046 |

Table 9 (continued). FRACTION OF DISSOLVED SULFIDE AS MOLECULAR $H_2S$ IN AQUEOUS SULFIDE SOLUTIONS OF LOW IONIC STRENGTH[a/]

| | Temperature, C | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 6.0 | .9111 | .9086 | .9060 | .9033 | .9006 | .8978 | .8949 | .8920 | .8890 | .8859 | .8827 | .8795 | .8762 |
| 6.1 | .8906 | .8876 | .8845 | .8813 | .8780 | .8747 | .8712 | .8677 | .8641 | .8605 | .8567 | .8529 | .8490 |
| 6.2 | .8661 | .8625 | .8588 | .8550 | .8511 | .8472 | .8431 | .8390 | .8348 | .8305 | .8261 | .8216 | .8170 |
| 6.3 | .8371 | .8328 | .8285 | .8241 | .8195 | .8149 | .8102 | .8054 | .8005 | .7956 | .7905 | .7853 | .7801 |
| 6.4 | .8032 | .7983 | .7933 | .7882 | .7830 | .7777 | .7723 | .7668 | .7612 | .7556 | .7498 | .7440 | .7380 |
| 6.5 | .7643 | .7587 | .7530 | .7472 | .7413 | .7353 | .7293 | .7231 | .7169 | .7106 | .7042 | .6977 | .6912 |
| 6.6 | .7203 | .7141 | .7077 | .7013 | .6948 | .6882 | .6815 | .6748 | .6679 | .6611 | .6541 | .6471 | .6400 |
| 6.7 | .6717 | .6648 | .6579 | .6509 | .6439 | .6368 | .6296 | .6223 | .6151 | .6077 | .6003 | .5929 | .5854 |
| 6.8 | .6191 | .6117 | .6044 | .5970 | .5895 | .5820 | .5745 | .5669 | .5593 | .5517 | .5440 | .5364 | .5287 |
| 6.9 | .5635 | .5559 | .5482 | .5406 | .5329 | .5252 | .5175 | .5098 | .5020 | .4943 | .4866 | .4789 | .4712 |
| 7.0 | .5063 | .4985 | .4908 | .4831 | .4754 | .4677 | .4600 | .4523 | .4447 | .4371 | .4295 | .4219 | .4144 |
| 7.1 | .4489 | .4412 | .4336 | .4261 | .4185 | .4110 | .4036 | .3962 | .3888 | .3815 | .3742 | .3670 | .3599 |
| 7.2 | .3928 | .3855 | .3782 | .3709 | .3638 | .3566 | .3496 | .3426 | .3357 | .3288 | .3220 | .3153 | .3087 |
| 7.3 | .3394 | .3326 | .3257 | .3190 | .3123 | .3057 | .2992 | .2928 | .2864 | .2801 | .2739 | .2678 | .2618 |
| 7.4 | .2899 | .2836 | .2773 | .2712 | .2651 | .2591 | .2532 | .2474 | .2417 | .2361 | .2306 | .2252 | .2198 |
| 7.5 | .2448 | .2392 | .2336 | .2281 | .2227 | .2174 | .2122 | .2071 | .2021 | .1971 | .1923 | .1875 | .1829 |

Table 9 (continued). FRACTION OF DISSOLVED SULFIDE AS MOLECULAR $H_2S$ IN AQUEOUS SULFIDE SOLUTIONS OF LOW IONIC STRENGTH[a/]

| | Temperature, C | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 7.6 | .2048 | .1998 | .1949 | .1901 | .1854 | .1808 | .1763 | .1718 | .1675 | .1632 | .1590 | .1549 | .1509 |
| 7.7 | .1698 | .1655 | .1613 | .1572 | .1531 | .1492 | .1453 | .1415 | .1378 | .1341 | .1306 | .1271 | .1237 |
| 7.8 | .1398 | .1361 | .1325 | .1290 | .1256 | .1222 | .1189 | .1157 | .1126 | .1096 | .1066 | .1037 | .1009 |
| 7.9 | .1143 | .1112 | .1082 | .1053 | .1024 | .09959 | .09685 | .09418 | .09158 | .08904 | .08657 | .08416 | .08181 |
| 8.0 | .09300 | .09042 | .08792 | .08547 | .08309 | .08076 | .07850 | .07629 | .07414 | .07205 | .07001 | .06803 | .06609 |
| 8.1 | .07531 | .07319 | .07112 | .06911 | .06714 | .06523 | .06338 | .06157 | .05981 | .05809 | .05642 | .05480 | .05322 |
| 8.2 | .06076 | .05902 | .05733 | .05568 | .05408 | .05252 | .05101 | .04953 | .04810 | .04670 | .04535 | .04403 | .04274 |
| 8.3 | .04888 | .04746 | .04608 | .04474 | .04344 | .04218 | .04095 | .03975 | .03859 | .03747 | .03636 | .03529 | .03425 |
| 8.4 | .03922 | .03807 | .03696 | .03587 | .03482 | .03379 | .03280 | .03183 | .03090 | .02998 | .02910 | .02824 | .02740 |
| 8.5 | .03141 | .03048 | .02958 | .02871 | .02786 | .02703 | .02623 | .02545 | .02470 | .02396 | .02325 | .02256 | .02189 |
| 8.6 | .02511 | .02436 | .02364 | .02294 | .02225 | .02159 | .02095 | .02032 | .01972 | .01913 | .01856 | .01800 | .01747 |
| 8.7 | .02005 | .01945 | .01887 | .01831 | .01776 | .01723 | .01671 | .01621 | .01573 | .01526 | .01480 | .01435 | .01392 |
| 8.8 | .01599 | .01551 | .01505 | .01460 | .01416 | .01373 | .01332 | .01292 | .01253 | .01216 | .01179 | .01144 | .01109 |
| 8.9 | .01274 | .01236 | .01199 | .01163 | .01128 | .01094 | .01061 | .01029 | .00998 | .00968 | .00939 | .00911 | .00883 |
| 9.0 | .01015 | .00984 | .00955 | .00926 | .00898 | .00871 | .00845 | .00819 | .00794 | .00770 | .00747 | .00725 | .00703 |

[a/] Sulfide solutions with ionic strength $\mu$ less than 0.01.

Table 10. MULTIPLICATION FACTORS FOR CONVERTING $H_2S$ CALCULATED FROM PUMEROY'S FACTORS FOR A "TYPICAL WATER SUPPLY" TO CORRESPONDING CONCENTRATIONS BASED ON THIS STUDY

| | Temperature, C | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 6.0 | 1.03 | 1.03 | 1.04 | 1.04 | 1.04 | 1.04 | 1.05 | 1.05 | 1.05 | 1.05 | 1.06 |
| 6.1 | 1.05 | 1.05 | 1.05 | 1.05 | 1.06 | 1.06 | 1.06 | 1.06 | 1.07 | 1.07 | 1.07 |
| 6.2 | 1.06 | 1.06 | 1.06 | 1.06 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| 6.3 | 1.07 | 1.07 | 1.07 | 1.07 | 1.08 | 1.08 | 1.08 | 1.08 | 1.09 | 1.09 | 1.10 |
| 6.4 | 1.08 | 1.08 | 1.09 | 1.09 | 1.09 | 1.09 | 1.10 | 1.10 | 1.10 | 1.10 | 1.11 |
| 6.5 | 1.09 | 1.09 | 1.10 | 1.10 | 1.11 | 1.11 | 1.12 | 1.12 | 1.12 | 1.12 | 1.13 |
| 6.6 | 1.11 | 1.11 | 1.12 | 1.12 | 1.13 | 1.13 | 1.14 | 1.15 | 1.16 | 1.16 | 1.17 |
| 6.7 | 1.14 | 1.14 | 1.15 | 1.16 | 1.16 | 1.16 | 1.17 | 1.17 | 1.18 | 1.18 | 1.19 |
| 6.8 | 1.16 | 1.16 | 1.17 | 1.17 | 1.18 | 1.18 | 1.20 | 1.20 | 1.21 | 1.21 | 1.22 |
| 6.9 | 1.19 | 1.19 | 1.20 | 1.20 | 1.22 | 1.22 | 1.23 | 1.23 | 1.25 | 1.25 | 1.26 |
| 7.0 | 1.22 | 1.22 | 1.24 | 1.24 | 1.25 | 1.25 | 1.26 | 1.25 | 1.27 | 1.26 | 1.28 |
| 7.1 | 1.24 | 1.24 | 1.25 | 1.25 | 1.27 | 1.27 | 1.29 | 1.29 | 1.31 | 1.31 | 1.33 |
| 7.2 | 1.28 | 1.28 | 1.30 | 1.30 | 1.32 | 1.32 | 1.34 | 1.33 | 1.35 | 1.35 | 1.37 |
| 7.3 | 1.32 | 1.32 | 1.34 | 1.34 | 1.35 | 1.35 | 1.36 | 1.35 | 1.37 | 1.36 | 1.38 |
| 7.4 | 1.34 | 1.33 | 1.35 | 1.34 | 1.36 | 1.36 | 1.38 | 1.37 | 1.39 | 1.39 | 1.41 |
| 7.5 | 1.36 | 1.35 | 1.36 | 1.37 | 1.38 | 1.37 | 1.39 | 1.38 | 1.39 | 1.38 | 1.40 |
| 7.6 | 1.36 | 1.35 | 1.37 | 1.36 | 1.38 | 1.37 | 1.39 | 1.38 | 1.40 | 1.40 | 1.42 |
| 7.7 | 1.37 | 1.36 | 1.38 | 1.38 | 1.40 | 1.40 | 1.43 | 1.43 | 1.46 | 1.46 | 1.49 |
| 7.8 | 1.41 | 1.41 | 1.45 | 1.45 | 1.47 | 1.45 | 1.47 | 1.46 | 1.47 | 1.47 | 1.49 |
| 7.9 | 1.44 | 1.43 | 1.45 | 1.44 | 1.46 | 1.45 | 1.47 | 1.46 | 1.49 | 1.49 | 1.51 |
| 8.0 | 1.45 | 1.44 | 1.47 | 1.46 | 1.48 | 1.47 | 1.49 | 1.48 | 1.50 | 1.50 | 1.52 |
| 8.1 | 1.46 | 1.45 | 1.48 | 1.47 | 1.49 | 1.47 | 1.49 | 1.48 | 1.50 | 1.49 | 1.51 |
| 8.2 | 1.46 | 1.45 | 1.47 | 1.46 | 1.47 | 1.46 | 1.47 | 1.46 | 1.47 | 1.46 | 1.49 |
| 8.3 | 1.44 | 1.43 | 1.44 | 1.43 | 1.45 | 1.44 | 1.47 | 1.46 | 1.49 | 1.49 | 1.52 |
| 8.4 | 1.44 | 1.44 | 1.47 | 1.47 | 1.48 | 1.46 | 1.48 | 1.46 | 1.47 | 1.46 | 1.48 |

Table 10 (continued). MULTIPLICATION FACTORS FOR CONVERTING $H_2S$ CALCULATED FROM POMEROY'S FACTORS FOR A "TYPICAL WATER SUPPLY" TO CORRESPONDING CONCENTRATIONS BASED ON THIS STUDY

| | Temperature, C | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 6.0 | 1.06 | 1.06 | 1.06 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.08 | 1.08 |
| 6.1 | 1.07 | 1.07 | 1.07 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 | 1.09 | 1.09 |
| 6.2 | 1.08 | 1.09 | 1.09 | 1.09 | 1.09 | 1.09 | 1.10 | 1.10 | 1.10 | 1.10 |
| 6.3 | 1.10 | 1.10 | 1.10 | 1.11 | 1.11 | 1.11 | 1.11 | 1.11 | 1.12 | 1.12 |
| 6.4 | 1.11 | 1.12 | 1.12 | 1.13 | 1.13 | 1.13 | 1.14 | 1.14 | 1.15 | 1.15 |
| 6.5 | 1.14 | 1.15 | 1.15 | 1.16 | 1.16 | 1.16 | 1.17 | 1.17 | 1.18 | 1.18 |
| 6.6 | 1.17 | 1.18 | 1.18 | 1.19 | 1.18 | 1.19 | 1.19 | 1.19 | 1.21 | 1.21 |
| 6.7 | 1.19 | 1.20 | 1.20 | 1.22 | 1.22 | 1.22 | 1.23 | 1.23 | 1.25 | 1.25 |
| 6.8 | 1.22 | 1.24 | 1.24 | 1.25 | 1.25 | 1.25 | 1.27 | 1.27 | 1.28 | 1.27 |
| 6.9 | 1.26 | 1.27 | 1.27 | 1.28 | 1.27 | 1.27 | 1.29 | 1.29 | 1.31 | 1.31 |
| 7.0 | 1.28 | 1.30 | 1.30 | 1.32 | 1.32 | 1.32 | 1.34 | 1.34 | 1.36 | 1.36 |
| 7.1 | 1.33 | 1.35 | 1.35 | 1.37 | 1.37 | 1.36 | 1.38 | 1.38 | 1.39 | 1.38 |
| 7.2 | 1.36 | 1.38 | 1.37 | 1.39 | 1.38 | 1.38 | 1.39 | 1.39 | 1.41 | 1.40 |
| 7.3 | 1.37 | 1.39 | 1.39 | 1.41 | 1.41 | 1.40 | 1.42 | 1.41 | 1.42 | 1.42 |
| 7.4 | 1.40 | 1.41 | 1.40 | 1.41 | 1.41 | 1.40 | 1.41 | 1.41 | 1.42 | 1.42 |
| 7.5 | 1.39 | 1.41 | 1.40 | 1.42 | 1.42 | 1.41 | 1.44 | 1.43 | 1.46 | 1.46 |
| 7.6 | 1.42 | 1.45 | 1.45 | 1.48 | 1.48 | 1.48 | 1.51 | 1.50 | 1.52 | 1.51 |
| 7.7 | 1.48 | 1.50 | 1.49 | 1.50 | 1.49 | 1.48 | 1.50 | 1.49 | 1.52 | 1.51 |
| 7.8 | 1.48 | 1.50 | 1.49 | 1.52 | 1.51 | 1.51 | 1.53 | 1.52 | 1.54 | 1.53 |
| 7.9 | 1.49 | 1.52 | 1.51 | 1.53 | 1.52 | 1.52 | 1.54 | 1.52 | 1.54 | 1.53 |
| 8.0 | 1.50 | 1.52 | 1.51 | 1.53 | 1.52 | 1.51 | 1.53 | 1.51 | 1.53 | 1.51 |
| 8.1 | 1.49 | 1.51 | 1.49 | 1.51 | 1.49 | 1.48 | 1.50 | 1.49 | 1.52 | 1.51 |
| 8.2 | 1.47 | 1.50 | 1.49 | 1.52 | 1.51 | 1.51 | 1.54 | 1.52 | 1.53 | 1.52 |
| 8.3 | 1.50 | 1.51 | 1.50 | 1.51 | 1.49 | 1.48 | 1.50 | 1.49 | 1.51 | 1.50 |
| 8.4 | 1.47 | 1.49 | 1.48 | 1.52 | 1.51 | 1.50 | 1.53 | 1.52 | 1.53 | 1.52 |

Table 10 (continued). MULTIPLICATION FACTORS FOR CONVERTING $H_2S$ CALCULATED FROM POMEROY'S FACTORS FOR A "TYPICAL WATER SUPPLY" TO CORRESPONDING CONCENTRATIONS BASED ON THIS STUDY

| | Temperature, C | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 8.5 | 1.44 | 1.42 | 1.44 | 1.43 | 1.45 | 1.44 | 1.46 | 1.46 | 1.49 | 1.48 | 1.51 |
| 8.6 | 1.44 | 1.43 | 1.46 | 1.46 | 1.47 | 1.46 | 1.48 | 1.47 | 1.49 | 1.49 | 1.52 |
| 8.7 | 1.45 | 1.44 | 1.47 | 1.46 | 1.49 | 1.48 | 1.52 | 1.52 | 1.57 | 1.57 | 1.60 |
| 8.8 | 1.50 | 1.50 | 1.55 | 1.55 | 1.56 | 1.54 | 1.54 | 1.53 | 1.54 | 1.52 | 1.54 |
| 8.9 | 1.51 | 1.49 | 1.50 | 1.49 | 1.51 | 1.50 | 1.52 | 1.51 | 1.53 | 1.53 | 1.54 |
| 9.0 | 1.49 | 1.48 | 1.50 | 1.50 | 1.51 | 1.49 | 1.50 | 1.49 | 1.51 | 1.49 | 1.52 |

determination. Therefore, it appears that the sulfide demand exhibited by some of the other test solutions is due to biological and other chemical processes and is not due appreciably to oxidation from dissolved oxygen.

The procedure used in preparing the sample for dissolved sulfide determination may have an effect on the calculated $H_2S$ concentration. The flocculation and centrifugation techniques for isolation of dissolved sulfide were not entirely satisfactory since in certain instances it appeared that not all of the suspended sulfides were removed and that during processing some decline in sulfide levels may also occur. For the types of samples prepared the millipore filtration technique is the best means of isolating and stabilizing dissolved sulfide. When this latter technique is used in conjunction with the factors defining the fraction of dissolved sulfide as $H_2S$ determined in this study, the calculated $H_2S$ concentration is very close to the $H_2S$ concentration determined by the direct method for all types of samples tested.

Table 10 (continued). MULTIPLICATION FACTORS FOR CONVERTING $H_2S$ CALCULATED FROM POMEROY'S FACTORS FOR A "TYPICAL WATER SUPPLY" TO CORRESPONDING CONCENTRATIONS BASED ON THIS STUDY

| | Temperature, C | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 8.5 | 1.50 | 1.51 | 1.50 | 1.52 | 1.52 | 1.51 | 1.54 | 1.53 | 1.57 | 1.56 |
| 8.6 | 1.51 | 1.55 | 1.54 | 1.59 | 1.59 | 1.59 | 1.62 | 1.60 | 1.61 | 1.59 |
| 8.7 | 1.58 | 1.59 | 1.57 | 1.58 | 1.56 | 1.54 | 1.57 | 1.55 | 1.57 | 1.56 |
| 8.8 | 1.53 | 1.55 | 1.54 | 1.56 | 1.55 | 1.55 | 1.57 | 1.55 | 1.56 | 1.54 |
| 8.9 | 1.53 | 1.54 | 1.52 | 1.54 | 1.53 | 1.51 | 1.54 | 1.52 | 1.55 | 1.54 |
| 9.0 | 1.50 | 1.53 | 1.52 | 1.55 | 1.55 | 1.55 | 1.57 | 1.53 | 1.56 | 1.54 |

RELATIONSHIP BETWEEN TEST pH AND SULFIDE TOXICITY TO THE FATHEAD MINNOW

A series of acute tests with fathead minnows to determine the relationship between test pH and the toxicity of sulfides was conducted at pH levels from about 6.5 to 8.7 and 20 C. A summary of the data and analysis for 16 bioassays of 96-hr duration is presented in Tables 14 through 17. Inspection of the data in Table 17 for composite tests grouped according to pH reveals that, with the exception of tests done at a pH of about 6.5, the ambient molecular $H_2S$ concentration required to give a 96-hr median tolerance limit (LC50) response decreased as the pH of the test solution increased. The 96-hr LC50's in ug/liter $H_2S$ ranged from 57.3 at pH 7.101 to 14.9 at pH 8.693. The anomalous results for the experiments performed at a pH of about 6.5 may be due to the interaction between sulfide and the relatively high $CO_2$ concentration in these test solutions. It is also quite feasible that the 96-hr LC50 values for $H_2S$ are relatively constant in test solutions over a pH range of about 6.5 to 7.1 and with the accompanying dissolved sulfide and $CO_2$ concentrations. Within the pH range of about 7.1 to 8.7, a 0.1 unit increase in pH was

Table 11. MULTIPLICATION FACTORS AT 25 C FOR CONVERTING $H_2S$ CALCULATED FROM FACTORS IN THE 1946 TO 1965 EDITIONS OF STANDARD METHODS TO CORRESPONDING CONCENTRATIONS BASED ON THIS STUDY

| pH | Factor | pH | Factor |
|---|---|---|---|
| 6.0 | 1.07 | 7.3 | 1.27 (1.46)[a/] |
| 6.2 | 1.10 | 7.4 | 1.46 |
| 6.4 | 1.14 | 7.5 | 1.48 |
| 6.5 | 1.19 | 7.6 | 1.56 |
| 6.6 | 1.20 | 7.7 | 1.55 |
| 6.7 | 1.24 | 7.8 | 1.59 |
| 6.8 | 1.29 | 7.9 | 1.60 |
| 6.9 | 1.31 | 8.0 | 1.59 |
| 7.0 | 1.37 | 8.2 | 1.60 |
| 7.1 | 1.37 (1.41)[a/] | 8.4 | 1.59 |
| 7.2 | 1.43 | 8.8 | 1.64 |

[a/] Factors in parenthesis apply to values originally published in Table IV of Pomeroy's 1941 work.

calculated by linear regression to result in a 2.7 ug/liter decrease in the molecular $H_2S$ 96-hr LC50 value. The linear regression correlation coefficient (r) was equal to -0.9945. From a practical standpoint the importance of this change in apparent toxicity of $H_2S$ is reduced since as pH is increased, the concentration of dissolved sulfide required to produce an acutely toxic solution is logarithmically increased. This change in concentration of dissolved sulfide, $HS^-$ ion, and molecular $H_2S$ concentration required to give the observed 96-hr LC50 toxic response at the pH of the test solution is shown in Figure 3. It should be emphasized that under relatively alkaline pH conditions the major portion of the dissolved sulfide consists of the $HS^-$ ion while only a small proportion exists as molecular $H_2S$.

When test pH within the range 6.5 to 8.7 for the composite fathead minnow sulfide bioassays at 20 C was compared with the log dissolved sulfide concentration at the 96-hr LC50 level, a positive linear relationship with a regression correlation coefficient of 0.9991 was calculated. The regression applying to dissolved sulfide levels ranging from 64-780 ug/liter as $H_2S$ is described by the equation:

$$\log Y = -1.278 + 0.477\ X \qquad (13)$$

where Y = 96-hr LC50 of dissolved sulfide as ug/liter $H_2S$
X = pH of test solution.

Table 12. DETERMINATION OF MOLECULAR $H_2S$ IN DIFFERENT WATERS[a] BY CALCULATION FROM THE TOTAL AND DISSOLVED SULFIDE CONCENTRATION AND BY A DIRECT TECHNIQUE

(concentrations expressed as ug/liter $H_2S$)

| Item | Deionized[b] | Well[b] | Well[c] | Fish aquarium[c] | 50% pond | Pond | Mississippi River[d] |
|---|---|---|---|---|---|---|---|
| Temperature, C | 20.0 | 19.8 | 19.8 | 20.0 | 19.8 | 19.8 | 20.1 |
| pH | 7.654 | 7.615 | 7.820 | 7.880 | 7.564 | 7.700 | 8.040 |
| Fraction dissolved sulfide as $H_2S$ | 0.176 | 0.204 | 0.128 | 0.168 | 0.209 | 0.162 | 0.0806 |
| Sulfide added | 221.4 | 184.7 | 181.2 | 174.5 | 338.6 | 519.3 | 317.3 |
| Total sulfide[e] | | | | | | | |
| Direct | 227.8 | 190.9 | 175.8 | 134.7 | 134.7 | 157.6 | 91.9 |
| Indirect | 220.5 | 168.1 | 174.7 | 114.9 | 139.7 | 154.5 | 93.7 |
| Dissolved sulfide after: | | | | | | | |
| Flocculation | 195.2 | 177.6 | 171.6 | 150.4 | 129.8 | 127.4 | 82.0 |
| Centrifugation | 212.7 | 178.2 | 162.5 | 123.8 | 135.9 | 137.1 | 72.1 |
| Filtration | 211.5 | 178.2 | 166.1 | 122.0 | 126.2 | 135.9 | 88.6 |
| $H_2S$ calculated from: | | | | | | | |
| Total sulfide - direct | 40.2 | 38.9 | 22.6 | 22.7 | 28.2 | 23.6 | 7.40 |
| - indirect | 38.8 | 34.2 | 22.4 | 19.3 | 29.2 | 25.0 | 7.55 |

Table 12 (continued). DETERMINATION OF MOLECULAR $H_2S$ IN DIFFERENT WATERS[a] BY CALCULATION FROM THE TOTAL AND DISSOLVED SULFIDE CONCENTRATION AND BY A DIRECT TECHNIQUE

(concentrations expressed as µg/liter $H_2S$)

| Item | Deionized[b] | Well[b] | Well[c] | Fish aquarium[c] | 50% pond | Pond | Mississippi River[d] |
|---|---|---|---|---|---|---|---|
| $H_2S$ calculated from dissolved sulfide after: | | | | | | | |
| Flocculation | 34.4 | 36.2 | 22.0 | 25.3 | 27.2 | 20.6 | 6.60 |
| Centrifugation | 37.5 | 36.3 | 20.8 | 20.8 | 28.4 | 22.2 | 5.80 |
| Filtration | 37.3 | 36.3 | 21.3 | 20.5 | 26.4 | 22.0 | 7.14 |
| $H_2S$ determined directly | 37.6 | 35.7 | 21.1 | 22.3 | 27.2 | 21.0 | 7.17 |

[a] Results represent one sample per test water. Test solutions prepared with less than 100% sample were diluted with deoxygenated well water.
[b] Deoxygenated.
[c] Oxygenated.
[d] Sample was taken below the Interstate 494 bridge, St. Paul, Minnesota.
[e] Direct colorimetric sulfide determination on sample or indirect determination following displacement and collection of sulfide on concentration column.

Table 13. DETERMINATION OF MOLECULAR $H_2S$ IN DIFFERENT EFFLUENTS[a] BY CALCULATION FROM THE TOTAL AND DISSOLVED SULFIDE CONCENTRATION AND BY A DIRECT TECHNIQUE

(concentrations expressed as ug/liter $H_2S$)

| Item | 50% sewage[b] | Sewage[b] | 10% Kraft paper processing | 10% hard board wood processing | Lagooned oil refinery |
|---|---|---|---|---|---|
| Temperature, C | 19.9 | 20.0 | 19.7 | 20.0 | 20.1 |
| pH | 7.732 | 7.478 | 7.974 | 7.738 | 7.856 |
| Fraction dissolved sulfide as $H_2S$ | 0.152 | 0.243 | 0.0936 | 0.150 | 0.118 |
| Sulfide added | 207.3 | 201.4 | 1056 | 990.8 | 538.3 |
| Total sulfide[c] | | | | | |
| Direct | 271.4 | 325.2 | 327.0 | 48.9 | 189.1 |
| Indirect | 277.7 | 303.5 | 336.5 | - | 190.0 |
| Dissolved sulfide after: | | | | | |
| Flocculation | 219.4 | 285.9 | 321.0 | 13.6 | 171.0 |
| Centrifugation | 259.9 | 304.0 | 282.3 | 24.6 | 158.9 |
| Filtration | 225.4 | 243.6 | 314.9 | 57.7 | 177.0 |
| $H_2S$ calculated from: | | | | | |
| Total sulfide - direct | 41.4 | 78.9 | 30.6 | 7.33 | 22.3 |
| - indirect | 42.2 | 73.7 | 31.5 | - | 22.4 |

Table 13 (continued). DETERMINATION OF MOLECULAR $H_2S$ IN DIFFERENT EFFLUENTS[a] BY CALCULATION FROM THE TOTAL AND DISSOLVED SULFIDE CONCENTRATION AND BY A DIRECT TECHNIQUE

(concentrations expressed as µg/liter $H_2S$)

| Item | 50% sewage[b] | Sewage[b] | 10% Kraft paper processing | 10% hard board wood processing | Lagooned oil refinery |
|---|---|---|---|---|---|
| $H_2S$ calculated from dissolved sulfide after: | | | | | |
| Flocculation | 33.4 | 69.4 | 30.0 | 2.04 | 20.2 |
| Centrifugation | 39.5 | 73.8 | 26.4 | 3.69 | 18.8 |
| Filtration | 34.3 | 59.1 | 29.5 | 8.64 | 20.9 |
| $H_2S$ determined directly | 34.6 | 61.1 | 25.3 | 6.81 | 19.0 |

[a] Results represent one sample per test effluent. Test solutions prepared with less than 100% sample were diluted with deoxygenated well water.

[b] Sewage samples from Minneapolis-St. Paul Metropolitan Sewage Treatment Plant were taken following secondary treatment.

[c] Direct colorimetric sulfide determination on sample or indirect determination following displacement and collection of sulfide on concentration column.

Table 14. SUMMARY OF TEST CONDITIONS IN SULFIDE BIOASSAYS AT DIFFERENT pH VALUES

| Test | pH | | Temp., C | | DO, mg/l | | Alkalinity, mg/l $CaCO_3$ | | | Free $CO_2$ in test solution[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Total | Phenol. | Bicarbonate | mg/l | mm Hg |
| 4C | 6.471 | .088 | 20.0 | .02 | 7.68 | .07 | 65.0 | - | 65.0 | | |
| 5C | 6.474 | .030 | 20.0 | .01 | 7.61 | .07 | 63.1 | - | 63.1 | | |
| 6C | 6.442 | .092 | 19.9 | .13 | 7.56 | .11 | 60.2 | - | 60.2 | | |
| Mean | 6.462 | .070 | 20.0 | .05 | 7.62 | .08 | 62.8 | - | 62.8 | 44.0 | 19.5 |
| 2B | 7.101 | .009 | 20.1 | .05 | 7.58 | .10 | 124.0 | - | 124.0 | | |
| 3B | 7.105 | .020 | 20.0 | .06 | 7.68 | .09 | 126.0 | - | 126.0 | | |
| 6B | 7.097 | .060 | 20.0 | .00 | 7.73 | .09 | 133.8 | - | 133.8 | | |
| Mean | 7.101 | .030 | 20.0 | .04 | 7.66 | .09 | 127.9 | - | 127.9 | 20.5 | 9.1 |
| 1A | 7.701 | .052 | 19.9 | .39 | 7.50 | .12 | 198.1 | - | 198.1 | | |
| 4A | 7.673 | .018 | 20.0 | .01 | 7.78 | .06 | 198.8 | - | 198.8 | | |
| 5A | 7.720 | .038 | 20.2 | .19 | 7.68 | .03 | 197.6 | - | 197.6 | | |
| Mean | 7.698 | .036 | 20.0 | .20 | 7.65 | .07 | 198.2 | - | 198.2 | 7.9 | 3.5 |
| 1B | 8.148 | .047 | 20.1 | .17 | 7.26 | .39 | 229.2 | - | 229.2 | | |
| 4B | 8.154 | .012 | 20.0 | .05 | 7.72 | .08 | 229.0 | - | 229.0 | | |
| Mean | 8.151 | .030 | 20.0 | .11 | 7.49 | .24 | 229.1 | - | 229.1 | 3.2 | 1.4 |

Table 14 (continued). SUMMARY OF TEST CONDITIONS IN SULFIDE BIOASSAYS AT DIFFERENT pH VALUES

| Test | pH | | Temp., C | | DO, mg/l | | Alkalinity, mg/l $CaCO_3$ | | | Free $CO_2$ in test solution[a/] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Total | Phenol. | Bicarbonate | mg/l | mm Hg |
| 2A | 8.445 | .025 | 20.1 | .05 | 7.53 | .13 | 238.2 | 4.0 | 234.2 | | |
| 3A | 8.416 | .011 | 19.9 | .05 | 7.57 | .09 | 238.2 | 3.5 | 234.7 | | |
| Mean | 8.430 | .018 | 20.0 | .05 | 7.55 | .11 | 238.2 | 3.8 | 234.4 | 1.7 | 0.75 |
| 2C | 8.693 | .010 | 20.1 | .21 | 7.39 | .12 | 242.9 | 9.3 | 233.6 | | |
| 3C | 8.707 | .011 | 20.1 | .03 | 7.47 | .14 | 243.4 | 9.5 | 233.9 | | |
| 6A | 8.679 | .020 | 20.1 | .07 | 7.60 | .06 | 241.6 | 8.3 | 233.3 | | |
| Mean | 8.693 | .014 | 20.1 | .10 | 7.49 | .11 | 242.6 | 9.0 | 233.6 | 1.0 | 0.44 |

[a/] Free $CO_2$ evaluated by nomographic method (APHA[1]). Assuming $K = H_2CO_3/P_{CO_2}$ and $-\log K = 1.41$ at 20 C, then 1 mg/liter $CO_2$ = 0.444 mm Hg $CO_2$ pressure (Stumm and Morgan[57], p. 148).

Table 15. DESCRIPTION OF FATHEAD MINNOWS USED IN SULFIDE BIOASSAYS AT DIFFERENT pH VALUES

| | Mean length, mm | | | Mean weight |
|---|---|---|---|---|
| Test | All fish | Survivors | Mortalities | survivors, g |
| 4C | 28.3 | 28.7 | 28.0 | 0.277 |
| 5C | 29.3 | 28.7 | 30.3 | 0.252 |
| 6C | 33.8 | 33.2 | 34.5 | 0.418 |
| Mean | 30.5 | 30.2 | 30.9 | 0.316 |
| 2B | 28.9 | 27.7 | 30.2 | 0.240 |
| 3B | 28.5 | 28.8 | 27.6 | 0.259 |
| 6B | 32.8 | 32.2 | 33.6 | 0.351 |
| Mean | 30.1 | 29.6 | 30.5 | 0.283 |
| 1A | 31.6 | 31.8 | 30.8 | 0.326 |
| 4A | 27.7 | 27.6 | 28.0 | 0.227 |
| 5A | 29.5 | 29.5 | 29.5 | 0.263 |
| Mean | 29.6 | 29.6 | 29.4 | 0.272 |
| 1B | 31.6 | 31.6 | 31.5 | 0.314 |
| 4B | 29.6 | 29.3 | 30.1 | 0.281 |
| Mean | 30.6 | 30.4 | 30.8 | 0.298 |
| 2A | 29.7 | 29.6 | 29.9 | 0.268 |
| 3A | 29.0 | 29.0 | 29.0 | 0.276 |
| Mean | 29.4 | 29.3 | 29.4 | 0.272 |
| 2C | 28.5 | 28.4 | 28.8 | 0.243 |
| 3C | 28.3 | 28.7 | 27.2 | 0.271 |
| 6A | 34.1 | 33.8 | 36.8 | 0.422 |
| Mean | 30.3 | 30.3 | 30.9 | 0.312 |

Table 16. BIOLOGICAL ASSAY BY THE BMDO3S PROBIT ANALYSIS METHOD[a] OF 96-HOUR FATHEAD MINNOW SULFIDE BIOASSAYS GROUPED ACCORDING TO TEST pH

| Average of grouped tests[b] | | Parameters in probit equation[c] Y = A + BX | | Chi-square statistic | Degrees of freedom | Log 96-hr LC50, µg/l $H_2S$ | Standard error of log LC50 |
|---|---|---|---|---|---|---|---|
| pH | Temp., C | A | B | | | | |
| 6.462 | 20.0 | -9.847 | 8.775 | 9.14 | 9 | 1.6920 | 0.1140 |
| 7.101 | 20.0 | -15.094 | 11.431 | 4.85 | 9 | 1.7579 | 0.0875 |
| 7.698 | 20.0 | -19.891 | 15.716 | 25.57[d] | 10 | 1.5839 | 0.0636 |
| 8.151 | 20.0 | -32.082 | 26.254 | 2.33 | 6 | 1.4125 | 0.0381 |
| 8.430 | 20.0 | -31.486 | 28.826 | 9.53 | 6 | 1.2657 | 0.0347 |
| 8.693 | 20.1 | -12.139 | 14.598 | 26.67[d] | 10 | 1.1741 | 0.0685 |

[a] See Dixon.[55]
[b] See table 14 for individual test conditions.
[c] A probit of 4.0, 5.0, and 6.0 corresponds to 16, 50, and 84% mortality, respectively, when Y is the maximum likelihood probit value and X is log $H_2S$ concentration in µg/liter.
[d] The $(chi)^2$ of the probit curve exceeds the value of $(chi)^2$ for P = 0.05, thus the data are significantly heterogeneous.

Table 17. SUMMARY OF LETHAL CONCENTRATION (LC) ANALYSIS FOR 96-HOUR FATHEAD MINNOW SULFIDE BIOASSAYS GROUPED ACCORDING TO TEST pH

(expressed as µg/liter $H_2S$)

| Average of grouped tests | | Fraction[a] dissolved sulfide | $H_2S$ 96-hr LC values | | | Slope[b] function, | 95% confidence limits for | Dissolved sulfide at LC50 | $HS^-$ at LC50 |
|---|---|---|---|---|---|---|---|---|---|
| pH | Temp., C | as $H_2S$ | 16% | 50% | 84% | S | $H_2S$ LC50 | level | level |
| 6.462 | 20.0 | 0.769 | 37.8 | 49.2 | 64.0 | 1.30 | 45.1-53.7 | 64.0 | 14.8 |
| 7.101 | 20.0 | 0.433 | 46.8 | 57.3 | 70.0 | 1.22 | 52.5-62.6 | 132.3 | 75.0 |
| 7.698 | 20.0 | 0.162 | 33.1 | 38.4 | 44.4 | 1.16 | 34.4-42.9[c] | 237.0 | 198.6 |
| 8.151 | 20.0 | 0.0637 | 23.7 | 25.8 | 28.2 | 1.09 | 24.4-27.2 | 405.0 | 379.2 |
| 8.430 | 20.0 | 0.0346 | 17.0 | 18.4 | 20.0 | 1.08 | 17.5-19.3 | 531.8 | 513.4 |
| 8.693 | 20.1 | 0.0191 | 12.8 | 14.9 | 17.5 | 1.17 | 13.5-16.4[c] | 780.1 | 765.2 |

[a] Obtained from Table 9 for average pH and temperature.

[b] S = (LC84/LC50 + LC50/LC16)/2.

[c] The $(chi)^2$ of probit curve exceeds value of $(chi)^2$ for p = 0.05, thus the data are significantly heterogeneous and a special means of calculating the confidence limits was employed (Litchfield and Wilcoxon[56]).

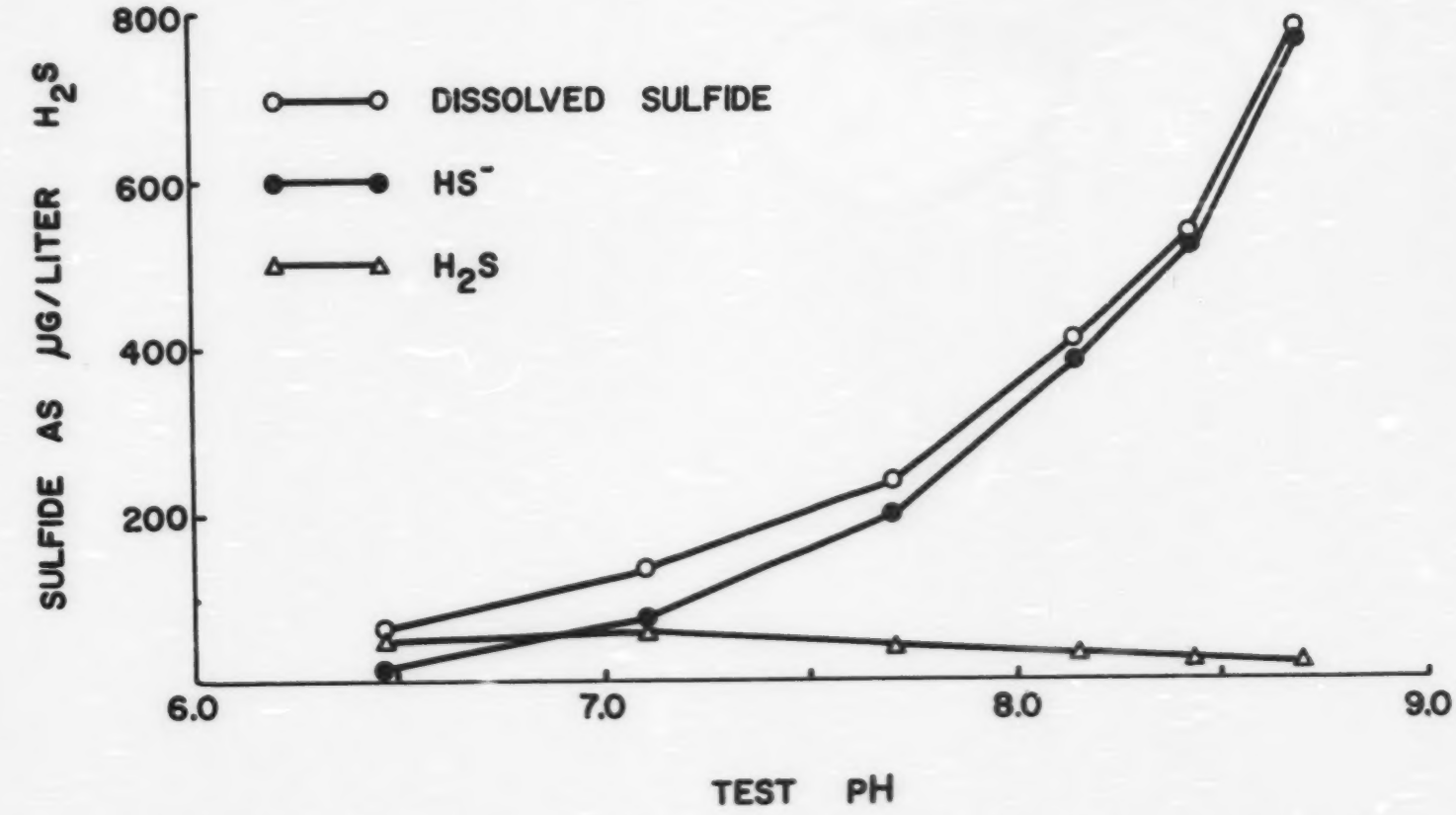


Figure 3. Relationship between test pH and dissolved sulfide, $HS^-$, and molecular $H_2S$ concentration at levels corresponding to the 96-hr LC50 for fathead minnows at 20 C.

## SECTION VI

## DISCUSSION

### DETERMINATION OF MOLECULAR $H_2S$ AND $K_1$ IONIZATION CONSTANTS OF $H_2S_{(aq)}$

The close agreement between the expression for $K_1$ of $(0.31 + 0.029\ T\ (C)) \cdot 10^{-7}$ derived from the literature (equation 4) and that of $(0.45 + 0.030\ T\ (C)) \cdot 10^{-7}$ defined during this study gives support to the validity of the methods and the accuracy of the equations proposed in this report. It can be demonstrated that what might be considered a slight change in $K_1$ can have a dramatic effect on the calculated percentage of dissolved sulfide as molecular $H_2S$. Therefore, it is critical that a correct expression for the relationship between $K_1$ and temperature be employed when calculating molecular $H_2S$ concentrations from dissolved sulfide. Equation (12),

$$pK_1 = 7.252 - 0.01342\ T\ (C), \qquad (12)$$

derived from data obtained in this study is believed to be an accurate expression for most freshwaters. The data employed in its calculation were obtained over the temperature range of 10 to 25 C. It is felt that the expression can be extended for use with temperatures ranging from at least 5 to 30 C with an acceptable loss in accuracy of calculated $pK_1$ values in the extrapolated temperature regions. Use of the expression at temperatures very far removed from those used in defining the equation is not recommended since the relationship between $pK_1$ and temperature may not be linear over extremes in temperature (Wright and Maass[6]).

The $K_1$ values determined in this study are "apparent" values and are not "true" ionization constants extrapolated to zero ionic strength. Since the values represent the average relationship determined from sulfide solutions prepared with deionized water and with a well water of relatively high alkalinity and total hardness, it is felt that the combined expression is applicable to most freshwaters of normal ionic strength ($\mu$ = 0.001 to 0.010). If no correction in the $pK_1$ temperature expression is made for ionic strength, it can be demonstrated that less than a 5% error in the calculated fraction of dissolved sulfide as molecular $H_2S$ would be realized at most combinations of temperature, pH, and ionic strength encountered in normal freshwaters. However, it is proposed that if the ionic strength of the solution is greater than about 0.01 but less than 0.10, the fraction of dissolved sulfide as molecular $H_2S$ should be calculated from the expression derived in the Appendix.

The accuracy of calculating molecular $H_2S$ concentrations from determined dissolved sulfide with factors derived in this study (Table 9), which correspond to the fraction of dissolved sulfide as molecular $H_2S$, was confirmed for many different types of freshwaters and industrial effluents. A technique utilized during this study also allows for the direct determination of molecular $H_2S$ at levels as low as a few µg/liter. However, this method may be of limited value since in many static test solutions sulfide could undergo oxidation during the $H_2S$ displacement and collection phase. The procedure could conceivably be used to monitor $H_2S$ in various waters when the gas stripping procedure is combined with a continuous-flow liquid phase sampler and a suitable sulfide detector system. A similar approach has been used by Garber, Nagano, and Wada[58] to measure $H_2S$ in sewer atmosphere and liquid.

## MODES OF TOXIC ACTION OF DISSOLVED SULFIDE TO FISH

It is generally recognized that the gill is the primary site designed for gas exchange between blood and water and that undissociated molecules will penetrate living tissues more readily than charged ions. The

pronounced toxicity of sulfide solutions in studies with fish has been assumed by numerous researchers, even though there is no conclusive direct experimental evidence, to be attributable to the action of undissociated molecular $H_2S$, varying with the pH and its concentration in solution, and not with the $HS^-$ or $S^{2-}$ ions. Recent recommendations of safe levels of sulfide have even been expressed in terms of concentrations of molecular $H_2S$ rather than dissolved sulfide.

Lloyd and Herbert[59] discuss the possible effect which $CO_2$ excreted via the gills of fish may have in shifting the $CO_2$-bicarbonate-carbonate chemical equilibrium and lowering of the pH of water in contact with the gills. These authors also proposed that the toxicity of ammonium salts is not strictly dependent on the pH value of the bulk solution but on the pH of the water at the gill surface. If Lloyd and Herbert's explanation is correct, the toxicity of sulfide solutions may be increased by respiratory depression of the pH in gills of fish due to excretion of $CO_2$ since the concentration of molecular $H_2S$ due to a conversion of $HS^-$ ions may be higher in the solution in contact with the gills than that surrounding the fish. According to their proposal, the pH at the gill surface can be calculated from the bicarbonate alkalinity, temperature, and free $CO_2$ concentration in the water, and the free $CO_2$ excreted by the gills of fish by use of the standard nomographic method and assuming that equilibrium of $CO_2$ hydration is rapid. The increase in the concentration of excreted $CO_2$ in the respiratory water (as mg $CO_2$/liter) is given by the following relation:

$$\text{Increase in } CO_2 = DO \times RQ \times \frac{\text{mol. wt. } CO_2}{\text{mol. wt. } O_2} \times \frac{P}{100} \qquad (14)$$

where DO = the dissolved oxygen concentration of the water in mg/liter
RQ = the respiratory quotient of the fish
P = the percentage of oxygen removed from the respiratory water by the fish.

Kutty[60] has determined that the respiratory quotient is essentially unity when the fish are spontaneously active and in near air-saturated water. Since the $CO_2$ is excreted along the surface of the lamellae, it is possible that there is a pH gradient formed in the gills. Lloyd and Herbert proposed that the average pH shift occurred for the condition when half the $CO_2$ was excreted and came to equilibrium with the carbonate system. When their proposed explanation was applied to the sulfide bioassay data in this study, it was calculated that the anomalous change in $H_2S$ toxicity with test solution pH could best be explained by their theory when assuming a respiratory quotient of unity and that the fathead minnow absorbs about 60% of the dissolved oxygen available through the gills. If a respiratory quotient of 0.8 is used, as was proposed by Lloyd and Herbert, then about 75% of the dissolved oxygen available through the gills would need to be absorbed to satisfy their theory.

The above proposed explanation for the toxicity to fish of weak acids and bases is indeed unique but for a number of reasons it may not be appropriate. First of all, it was assumed by Lloyd and Herbert[59] in their calculations that the utilization of oxygen in the water passing over the gills of rainbow trout is about 80%. Recent studies have shown that the percentage utilization of oxygen is variable between fish and for a given fish under different conditions. According to information presented in a review by Shelton,[61] almost all of the reported utilization values are less than 80% under near ideal environmental conditions. During the stress occurring in an acute toxicity bioassay, it is not unreasonable to assume that the utilization might even be considerably less than the 60% value necessary to justify the pH drop at the gill theory for the sulfide bioassays.

A second and most important criticism of this theory arises when one examines the manner in which $CO_2$ is excreted at the gills and the amount which is excreted. According to Randall,[62] the rate at which $CO_2$ is exchanged across the gills depends on the dimensions of the epithelium, the concentration gradient, and the diffusion coefficient of $CO_2$. These factors are such that the expected changes in $CO_2$ tension in the inspired and expired water passing over the gills are small and at most a few mm Hg. To satisfy Lloyd and Herbert's explanation of the sulfide bioassay data, the increase in $CO_2$ at the gill surface when assuming a 60% utilization would need to be about 6.2 mg/liter or 2.8 mm Hg. Randall[62] also stated that the mechanism for the excretion of $CO_2$ from the cells of freshwater teleosts includes the conversion to bicarbonate of some of the $CO_2$ in the blood by carbonic anhydrase located in the gill epithelium. Therefore, along with the free $CO_2$ entering the water, bicarbonate passes across the gill epithelium by an exchange diffusion mechanism which involves chloride. Stumm and Morgan[57] indicate that the hydration/dehydration reaction

$$CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3 \qquad (15)$$

proceeds very slowly and the establishment of the hydration equilibrium at pH values near 7 requires a finite time on the order of many seconds. It should also be noted that the formation of $CO_2$ from the bicarbonate actively diffusing out of the gill epithelium is slow. Therefore, since a volume of water is generally considered to be in contact with the gill epithelium for less than 2 seconds, and the hydration of $CO_2$ and formation of $CO_2$ from bicarbonate in water is on the order of many seconds, the major portion of the rise in $P_{CO_2}$ and ultimate pH shift at equilibrium should occur after the water has left the respiratory surface. This process tends to maintain the necessary $P_{CO_2}$ gradient between blood and water.

Molecular species are known to penetrate membranes more readily than charged ions. If it is assumed that molecular $H_2S$ is the major internal

toxic sulfide species, then an explanation for the observed relationship between test solution pH and sulfide toxicity may include the penetration of the gill epithelium mainly by molecular $H_2S$ accompanied by a change in blood and intracellular pH in accord with ambient $CO_2$ tensions. Albers[63] has stated that the relationship between fish blood pH and log $P_{CO_2}$ is linear and may differ in slope, depending on buffer capacity and absolute values with different species. In his review it can be seen in Figure 11 (p. 197) that for Cyprinus carpio, as the blood $P_{CO_2}$ is increased from about 2 to 12 mm $H_g$, the pH decreases from about 7.9 to 7.4. The blood pH of the tested fathead minnows probably decreased with increasing ambient $CO_2$ tensions (decreasing test pH). The intracellular pH is generally lower than that of the blood. Therefore, the observed increase in molecular $H_2S$ toxicity with increasing test solution pH may partially be explained by the difference in the degree of ionization of molecular $H_2S$ following penetration of the gill epithelium for fish exposed to different test pH values. A similar expanation for the effect of ambient $CO_2$ tensions on the toxicity of ammonia was proposed by Warren and Schenker.[64] A change in the permeability of the gill to molecular $H_2S$ may also contribute to the observed apparent change in $H_2S$ toxicity. However, the nearly fourfold change in penetration rate over the pH range of 7.1 to 8.7 necessary to account entirely for the change in $H_2S$ LC50 values from 57.3 to 14.9 µg/liter is not very likely.

Among its various modes of toxic action, poisoning by sulfide species includes the formation of metal complexes. It has been documented (White, Handler, and Smith[65]) that sulfide, including its anionic species, can inhibit certain enzymes by formation of complexes with essential metal ions contained in the enzyme. Sulfide species are known to inhibit iron-containing enzymes such as peroxidase and catalase. They also bind to the ferric ion of cytochrome oxidase and thereby inhibit $O_2$ metabolism. The sulfmethemoglobin formed from the combination of sulfide species with the ferric ion of methemoglobin cannot be further metabolized and remains until the cell is phagocytized.

If dissolved sulfide species other than molecular $H_2S$ can penetrate the gills and since they can inhibit certain enzymes, the toxicity of sulfide solutions to fish should not be entirely related to the ambient molecular $H_2S$ level but should be more closely correlated with the internal total dissolved sulfide concentration. The negative relationship between pH and molecular $H_2S$ LC50 levels can possibly be explained by assuming that the $HS^-$ ion penetrates the gill epithelium, though presumably to a much lesser extent than molecular $H_2S$, and contributes to the toxicity of sulfide solutions to a greater degree as the pH increases. Therefore, the results obtained in this study demonstrated that the acute toxicity to fathead minnows of sulfide solutions does not depend entirely on the concentration of ambient molecular $H_2S$, but that the $HS^-$ ion may contribute to a much lesser extent to the toxicity of these solutions. However, definition of the relative toxicity of these two dissolved sulfide species awaits further physiological and toxicological evaluation.

## SECTION VII

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## SECTION VIII

## APPENDIX

### DERIVATION OF AN EQUATION TO CALCULATE THE FRACTION OF DISSOLVED SULFIDE AS MOLECULAR $H_2S$ WHEN THE IONIC STRENGTH OF THE SOLUTION IS LESS THAN 0.10

The dissociation of $H_2S$ in aqueous solution can be represented by:

$$H_2S_{(aq)} \underset{\leftarrow}{\overset{K_1}{\rightleftharpoons}} H^+ + HS^- \underset{\leftarrow}{\overset{K_2}{\rightleftharpoons}} 2H^+ + S^{2-} \qquad (1)$$

The equilibrium constants $K_1$ and $K_2$ are given by:

$$K_1 = \frac{a_{H^+} \cdot a_{HS^-}}{a_{H_2S_{(aq)}}} \text{ and } K_2 = \frac{a_{H^+} \cdot a_{S^{2-}}}{a_{HS^-}} \qquad (16)$$

where $K_1 = 10^{-7}$ at 20 C

$K_2 = 10^{-13.5}$ at 20 C

$a_{H_2S_{(aq)}}$ = activity of undissociated molecular $H_2S$ dissolved in water

$a_{HS^-}$ = activity of hydrosulfide ion

$a_{S^{2-}}$ = activity of sulfide ion

$a_{H^+}$ = activity of hydrogen ion.

The concentration of sulfide species is determined by the first and second dissociation constants and the equilibrium solution conditions. By calculation it can be demonstrated that when the pH is less than about 11 the second equilibrium constant $K_2$ is so small that it and the presence of sulfide ions ($S^{2-}$) can be neglected in equilibrium calculations in the following discussion.

The total concentration of dissolved sulfide species in solution may be expressed by:

$$\text{Dissolved sulfide} = [DS] = [H_2S_{(aq)}] + [HS^-]. \tag{17}$$

Since

$$a_A = [A] \cdot f_A$$

where $a_A$ = activity of the species A
$[A]$ = molar concentration
$f_A$ = dimensionless number called the activity coefficient

then

$$K_1 = \frac{a_{H^+} \cdot [HS^-] \cdot f_{HS^-}}{[H_2S_{(aq)}] \cdot f_{H_2S_{(aq)}}} \tag{18}$$

From the dissolved sulfide expression (equation 17):

$$[HS^-] = [DS] - [H_2S_{(aq)}] \tag{19}$$

$$K_1 = \frac{a_{H^+} \cdot [DS] - [H_2S_{(aq)}] \cdot f_{HS^-}}{[H_2S_{(aq)}] \cdot f_{H_2S_{(aq)}}}$$

$$K_1 = \frac{\left(a_{H^+}\ f_{HS^-}\ [DS]\right) - \left(a_{H^+}\ f_{HS^-}\ [H_2S_{(aq)}]\right)}{[H_2S_{(aq)}] \cdot f_{H_2S_{(aq)}}}$$

$$K_1\ [H_2S_{(aq)}]\ f_{H_2S_{(aq)}} = \left(a_{H^+}\ f_{HS^-}\ [DS]\right) - \left(a_{H^+}\ f_{HS^-}\ [H_2S_{(aq)}]\right)$$

$$\left(K_1\ [H_2S_{(aq)}]\ f_{H_2S_{(aq)}}\right) + \left(a_{H^+}\ f_{HS^-}\ [H_2S_{(aq)}]\right) = a_{H^+}\ f_{HS^-}\ [DS]$$

$$[H_2S_{(aq)}]\left(K_1\ f_{H_2S_{(aq)}} + a_{H^+}\ f_{HS^-}\right) = a_{H^+}\ f_{HS^-}\ [DS]$$

$$[H_2S_{(aq)}] = \frac{a_{H^+}\ f_{HS^-}\ [DS]}{K_1\ f_{H_2S_{(aq)}} + a_{H^+}\ f_{HS^-}}$$

$$[H_2S_{(aq)}] = \frac{a_{H^+}\ f_{HS^-}}{K_1\ f_{H_2S_{(aq)}} + a_{H^+}\ f_{HS^-}} \cdot [DS] \qquad (20)$$

In freshwaters of relatively low ionic strength, it can be assumed that for the molecular species $H_2S_{(aq)}$

$$f_{H_2S_{(aq)}} = 1 \qquad (21)$$

Thus

$$[H_2S_{(aq)}] = \frac{a_{H^+}\ f_{HS^-}}{K_1 + a_{H^+}\ f_{HS^-}} \cdot [DS] \qquad (22)$$

This expression may also be written in concentration units where C corresponds to μg/liter as $H_2S$:

$$C_{H_2S_{(aq)}} = \frac{a_{H^+} f_{HS^-}}{K_1 + a_{H^+} f_{HS^-}} \cdot C_{DS} \qquad (23)$$

By definition $a_{H^+} = \text{antilog}_{10}\ (-pH)$ when standard buffers prepared according to the National Bureau of Standards recommendations are used to standardize the pH meter. Therefore:

$$C_{H_2S_{(aq)}} = \frac{\text{antilog}\ (-pH) \cdot f_{HS^-}}{K_1 + \text{antilog}\ (-pH) \cdot f_{HS^-}} \cdot C_{DS} \qquad (24)$$

From the above expression, it is apparent that the concentration of molecular $H_2S$ in an aqueous solution of known dissolved sulfide concentration can be calculated for various solution pH and temperature values by knowing the following equilibrium solution parameters:

$K_1$ - the relationship between the equilibrium constant for the first dissociation of $H_2S_{(aq)}$ in aqueous solution with temperature. From research performed in this study this relationship can for all practical purposes be defined by equation (12)

$$pK_1 = 7.252 - 0.01342\ T\ (C).$$

$f_{HS^-}$ - the activity coefficient of the $HS^-$ ion which varies with solution ionic strength and temperature.

In dilute solution of electrolytes, the individual ion activity coefficient is given by the extended Debye-Hückel expression:

$$f_{HS^-} = \text{antilog}\left[ - \frac{A \cdot Z_{HS^-}{}^2 \cdot \sqrt{I}}{1 + a^{\circ}_{HS^-} \cdot B\sqrt{I}} \right] \qquad (25)$$

where $A$ = a constant

$B$ = a constant

$a^\circ$ = a constant

$Z_{HS^-}$ = charge of the $HS^-$ ion equal to 1

$I$ = ionic strength of the solution.

Garrels and Christ[1] give the value of $a^\circ_{HS^-}$ as $3.5 \times 10^{-8}$ and give tables of data for A and B which for the temperature range of 0 to 30 C can be expressed by:

$$A = 0.4880 + 0.0082\ T$$

$$B = (0.3241 + 0.00016\ T) \cdot 10^8$$

where $T$ = temperature in C.

Thus

$$f_{HS^-} = \text{antilog}\left[-\frac{(0.4880 + 0.00082\ T)\ \sqrt{I}}{1 + \sqrt{I}\ (1.134 + 0.00056\ T)}\right] \qquad (26)$$

Therefore, the fraction of dissolved sulfide (DS) as molecular $H_2S$ is given by:

$$\frac{C_{H_2S(aq)}}{C_{DS}} = \frac{f}{f + k} \qquad (27)$$

where $f = \text{antilog}(-pH) \cdot \text{antilog}\left[-\frac{(0.4880 + 0.00082\ T)\sqrt{I}}{1 + \sqrt{I}\ (1.134 + 0.00056\ T)}\right]$ (28)

$k = \text{antilog} - [7.252 - 0.01342\ T]$ (29)

pH = final pH of the solution

T = temperature of the solution in C

I = ionic strength of the solution.

Much of the information used in this derivation was previously included in an information report by Clarke.[2]

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